

For a human being, the chance of experiencing at least one seizure during a lifetime is approximately 2-5%. Seizures are defined as transient changes of behavior due to inappropriate, synchronous and rhythmic firing of central nervous system neurons. Often they originate from a well-defined brain area. In patients with a so-called epileptic focus, experienced neurologists can make a good educated guess on the localization of the focus based on observation of the seizure. However, descriptions of seizures in both preclinical and clinical research have been of a qualitative nature. The literature provides detailed descriptions of the symptoms of seizures, but these observations are not quantified. Quantitative estimation is of interest, not only for a more detailed characterization of seizures but also for measurement of the effects of anti-epileptic drugs. Without quantitative estimation, the only information available is whether the seizure was suppressed yes or no without any gradation. Using The Observer, we counted the occurrence of seizure components in rats with different types of electrically-induced seizures. In addition, the relation between drug concentration and suppression of these seizure components was estimated for several anti-epileptic drugs.

#### **METHODOLOGICAL ISSUES**

The seizures studied have a time span smaller than 2 s with a large number of behaviors occurring in a fast sequence and sometimes simultaneously. Under these conditions it is impossible to obtain reliable counts of seizure components with the bare eye. For that reason we decided to digitize video images on-line during the experiment and save them on disk. From these files the seizures were analyzed in The Observer Video-Pro with the focal sampling method. The video player was used for playback at different speeds and quick browsing through the video images. This proved to be very useful during scoring of the seizure components because it was necessary to view each seizure at least twice at different speeds. In addition, we needed to determine inter- and intra-observer validity. The Observer enabled us to easily retrieve video images with the corresponding observational data files to refine our definitions of each seizure component.

#### **METHODS**

##### *Counting seizure components*

Electrodes for electrical stimulation were implanted on the cortex of ten rats at three different positions in each animal. From each electrode position, seizures were elicited with 50 Hz bipolar pulses increasing linearly in magnitude. From all behaviors observed, four seizure components of interest were selected for analysis. These were eye closure, forelimb clonus, forelimb tonus and head jerk. After scoring, gross data errors in the observational files were detected using time-event plots generated with The Observer. The Elementary Statistics module was used to create a report of all observed frequencies for export to Excel.

##### *Determination of drug effects*

Midazolam or saline were infused intravenously into another 20 rats and blood samples were taken at intervals for determination of plasma concentrations of midazolam. Simultaneously, the rats received repeated cortical stimulation as described above. An advanced statistical model was developed to relate drug concentration to the occurrence of each seizure component. Specifically, a nonlinear mixed-effects model for Poisson-distributed data was used.

## RESULTS

### *Quantification of seizure components*

Stimulation of different parts of the cortex resulted in different seizure patterns. The number of times a seizure component was observed per stimulation differed significantly between electrode positions.

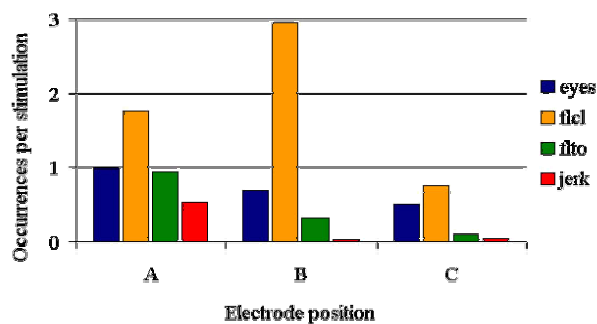


Figure 1. Seizure patterns observed after stimulation of different parts of the cortex.

### *Concentration dependency*

After infusion of the anti-epileptic drug midazolam, the occurrence of all seizure components was suppressed in a concentration-dependent manner. Estimates of the baseline occurrence and the potency of midazolam with respect to each seizure component were obtained.

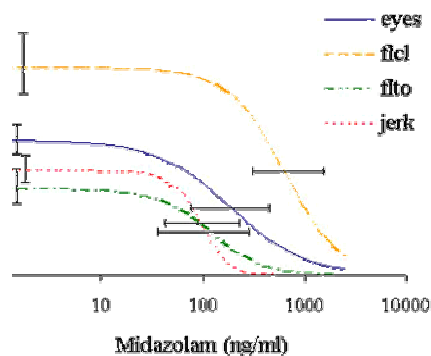


Figure 2. Estimates of the dependency of seizure components on drug concentration. Each curve represents the predicted occurrence of one seizure component, with error bars indicating s.e.m. A significant difference in baseline values was found, but the potency of midazolam did not differ between the seizure components studied.

## CONCLUSIONS

In epilepsy research, quantification of seizure components is not commonly performed. We have shown that stimulation of different brain areas results in seizures, which are qualitatively similar, but differ quantitatively. Furthermore, it is possible to quantify the suppressive effects of anti-epileptic drugs on seizure components. In principle the present technique can be used in any experimental epilepsy model and in patients with epilepsy. Also, this technique enables us to evaluate the effects of any anti-epileptic drug, irrespective of its mechanism of action. For that reason, future experiments using The Observer will be directed to evaluating the effects of combinations of anti-epileptic drugs.



Figure 3. Experiment setup with cages with rats and the monitor receiving the image from one of the cages. On the left the stimulation generator and video recorder.

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