EVALUATION OF PROCONVULSANT RISK USING TESTS EVALUATING SPONTANEOUS AND PROVOKED CONVULSIONS
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INTRODUCTION
Despite the fact that the core battery for safety pharmacology does not include studies on pro-convulsant risk, this assessment remains an important step in the drug development process. The aim of the study was to evaluate the utility of different tests, either based on the observation of spontaneous convulsions or the facilitation of provoked convulsions, in the evaluation of the pro-convulsant profiles of drugs. Theophylline, a well-known proconvulsant substance in humans, was used as a positive control and evaluated in the rat.

METHODS
- **Treatment**: Theophylline was dispersed in 0.2% hydroxypropylmethylcellulose in physiological saline and was administered intraperitoneally, either immediately or 30 minutes before the test.
- **Irwin test**: Behavioral symptoms were evaluated at different time-points (15, 30, 60, 120 and 180 minutes) after administration of theophylline.
- **Video-EEG monitoring**: Rats were implanted with a telemetric device introduced into the peritoneal cavity and the biopotential positive and negative leads were soldered to two depth electrodes inserted stereotaxically into the hippocampus (CA1 region). After recovery, rats were placed individually within their home cage close to a telemetry receiver and were continuously video-recorded during 120 minutes after administration of theophylline. All generated data were acquired and analysed using the EMKA Technologies softwares (IOX version 2.8.2.10 and ECG-Auto version 2.6.0.20).
- **Electroconvulsive Shock Threshold (ECST)**: Rats were administered electroconvulsive shock (rectangular current: 0.6 ms pulse width, 1.5 s duration, 200 Hz) via earclip electrodes connected to a constant current shock generator. The first animal of each group was exposed to 30 mA of ECS. Then, the intensity of the stimulation was decreased or increased for the next animal tested, based on the response of the previous animal (up-and-down method).
- **Pentylentetrazole (PTZ)-induced convulsion test**: rats were injected with PTZ (75 mg/kg s.c.) and were immediately placed in individual macrolon cages. The occurrence of convulsions and death were noted over a 30-minute period after PTZ administration.

RESULTS

**Fig. 1 (n=6/group):** Theophylline up to 64 mg/kg dose- and time-dependently increased sniffing and motor activity. At 128 mg/kg, a single rat displayed stereotypes and tremor followed by convulsions and death. Signs of excitation increased sniffing and motor activity. At 128 mg/kg, a single rat displayed stereotypes and tremor followed by convulsions and death. Signs of excitation increased sniffing and motor activity. At 128 mg/kg, a single rat displayed stereotypes and tremor followed by convulsions and death. Signs of excitation increased sniffing and motor activity.

**Fig. 2 (n=12/group):** a) Seizure activity accompanied with convulsive symptoms were observed in 3 rats at 128 mg/kg. Paroxysmal activity was detected in 2 rats in the absence of any clear behavioral symptoms. b) EEG after administration of theophylline at 128 mg/kg: 1. Normal EEG. B. Train of spikes over a period of 2 seconds in absence of convulsive symptoms. 2. Seizure activity during clonic convulsions.

**Fig. 3 (n=10/group):** Theophylline dose-dependently decreased the intensity to induce tonic convulsions, as compared with vehicle controls.

**Fig. 4 (n=20/group):** Theophylline dose-dependently increased the number of rats displaying clonic and tonic convulsions and the number of rats that died, as compared with vehicle controls.

CONCLUSION
- Evaluation of spontaneous convulsions provides information on the therapeutic window of a drug and the translational value of this approach is increased by the use of video-EEG. Tests based on provoked convulsions further complement the evaluation since they mimic high risk situations. Measurement of both spontaneous and provoked convulsions improves the evaluation of the pro-convulsant risk of novel pharmacological substances, critically important in the drug development process.