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Inter- and intraobserver agreement of seizure behavior scoring in the amygdala kindled rat

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ABSTRACT

Introduction: The Racine scale is a 5-point seizure behavior scoring paradigm used in the amygdala kindled rat. Though this scale has been applied widely in experimental epilepsy research, studies of reproducibility are rare. The aim of the current study was, therefore, to assess its interobserver variability and intraobserver variability.

Material and methods: A video database set was acquired in the course of amygdala kindling of 67 Wistar rats. Six blinded observers received scoring instructions and then viewed a set of 15 random videos (session #1). Next, each observer scored 379 to 1048 additional videos (session #2) and finally scored the same set of 15 videos again (session #3). Scores included the occurrence of seizures (yes or no), the total seizure time (start of stimulus until the absence of seizure behavior), and the highest Racine stage. Interobserver variability and intraobserver variability were assessed in and between sessions #1 and #3 using a 2-way mixed intraclass correlation or Cohen's kappa depending on the variable.

Results: Interobserver agreement in session #1 was 0.664 for seizure occurrence, 0.861 for total seizure time, and 0.797 for the highest Racine stage. In session #3, interobserver agreement on seizure occurrence declined to 0.492, total seizure time declined to 0.625, and agreement for the highest Racine stage was 0.725. Interobserver agreement was scored insufficiently on focal R2 seizures in both sessions (0.287 and 0.182). Intraobserver agreement reached >0.80 agreement for seizure occurrence, highest seizure score, and total seizure time in 3 out of 4 observers. Racine's scale stage 2 seizure scores were only 0.135 in one observer but 0.650, 0.810, and 0.635 in the other observers.

Discussion and conclusion: Overall, interobserver agreement and intraobserver agreement in scoring with Racine's scale were adequate. However, because interobserver agreement declined after a period of individually scoring videos, we suggest periodic repetition of the standardized instruction in the course of evaluating videos in order to ensure reproducible results.

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1. Introduction

In 1972, Ronald J. Racine published his landmark classification system of seizure behavioral stages in the amygdala kindled (AK) rat model [1]. Since then, the Racine scale has become the mainstay method of seizure classification in many different animal models.

The AK rat is a model widely used in epilepsy research. Through a surgically implanted electrode, the rat amygdala is stimulated electrically for a few seconds each day. In time, animals display a series of typical, stimulation-induced behavioral stages that coincide with epileptiform activity as registered by electroencephalography (EEG) [1,2]. The behavioral stages resemble the generalized tonic–clonic features often seen in patients with temporal lobe epilepsy [1]. In AK rats, seizures usually start with mild focal symptoms, mostly facial and/or oral clonus, that quickly become more pronounced. After a phase of unilateral forelimb clonus, seizures generalize, characterized by bilateral forelimb clonus and, eventually, by rearing and a loss of balance due to clonus in all limbs [3].

Although the Racine stages are well described, they are also very concise [1]. This leads to classification of seizure behavior being hampered by a heterogeneous display.

Abbreviations: AK, Amygdala kindling; EEG, Electroencephalography; ICC, Intraclass correlation.

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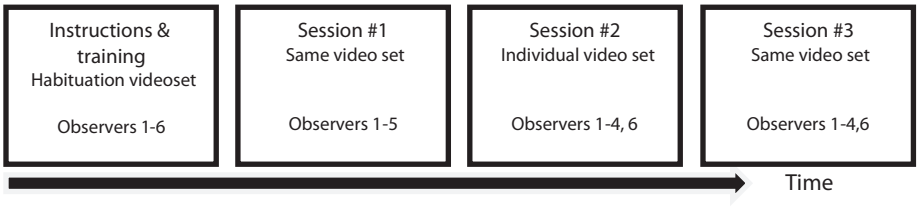

Fig. 1. Chronological scoring of videos for each observer. After an instruction session, observers viewed a subset of videos for the first time, followed by individual scorings of a unique subset of videos. Hereafter, the same video subset as in session #1 was scored again.

Furthermore, behavioral observations are subjective because of the knowledge and experience of the observer, potentially causing variability in the classification of rat behavior [4]. Even with an established scoring paradigm, seizure behavior classification can be challenging because of the unpredictable and variable nature of seizures, as is known from human studies [5,6].

It is, therefore, surprising that very little research has been performed on the observer reproducibility of Racine's scale in the AK rat. As such, the reliability of this scoring system is unknown.

The aim of the current study was, therefore, to analyze interobserver agreement and intraobserver agreement in behavioral scoring of seizures in AK rats. For this purpose, we used the Racine scale to investigate observation parameters applying to different aspects of behavioral seizures.

2. Materials and methods

2.1. Animal treatment

Videos were obtained from 67 female Wistar rats that were used in a kindling experiment conducted at our facility. The performed procedures in this study were the following: amygdala kindling (twice daily, 400 μA, 60 Hz, 2 s), ADT determination (20% step procedures), and ADT determination after phenytoin injections.

A single video file contained a registration of a single stimulus run. During the runs, rats were housed in a 50 × 50 × 50 custom made transparent Plexiglas box. Seizures were recorded by a hand-held digital camera which followed each rat's position. Video recordings of all stimulation sessions were uploaded to a single video database after recording.

2.2. Observers

Prior to this study, observers were completely unexperienced with behavioral animal scoring in general and the Racine scale in particular. An exception was observer #1, who was responsible for the instruction of all other observers and the construction of the instruction video set and, thus, had more experience with rat behavioral scoring. Before scoring began, all observers received an oral instruction together with 24 videos with typical examples of all 5 Racine stages (Fig. 1).

After the instruction, the observers reviewed video subsets in a fixed order. For the first video set, 15 videos were randomly selected from the database and scored by observers #1 to #5 (session #1). After scoring this subset, observers viewed a number of other videos, which varied per observer (session #2). Observer #1 viewed the most videos (1048) followed by observer #6 (742), observer #3 (559), observer #2 (535), and observer #4 (379). Observer #5 only completed session #1. For the third scoring session, observers #1 to #4 and #6 revaluated the video subset of session #1. To ensure that our number of observers and observations were sufficient, we used a paradigm by Walter et al. [7].

2.3. Video analysis

Scoring was performed based on the classical Racine scale [1] which was interpreted by observer #1 in making the instruction video set. We scored as follows: 0 = no behavioral effect, R1 = mouth and facial movements, R2 = head nodding, R3 = forelimb clonus, R4 = rearing, and R5 = rearing and falling. Observers did not assess the R1 stage.

For each stimulus-induced seizure, the following parameters were measured by the observers from the start of a stimulus — seizure occurrence outside the 2-second stimulus time (yes or no), the highest Racine score, and the total seizure time between R1 and R5 in seconds including latency time until onset of seizure behavior. The generalized seizure time was measured in seconds between the start of stimulus and the end of R4 or R5 including latency. Finally, the duration of each Racine stage from the start of stimulus was measured in seconds including latency time.

2.4. Data analysis

Interobserver variability was assessed for sessions #1 and #3 for all observers who participated in that session. Agreement was determined separately using a 2-way mixed, consistency single-measures intraclass correlation (ICC) for all variables to assess interobserver agreement. Since ICC has no unit of measurement, values are reported as means with a 95% confidence interval (95% CI) and interpreted using a paradigm developed by Cicchetti (Table 1) [8].

The intraobserver variability analysis focused on the difference in scoring between sessions #1 and #3 for observers #1 to #4. For noncontinuous data, the marginal distribution of the data indicated no prevalence or bias problems, making Cohen's kappa the appropriate method of analysis [9]. For interpretation, a well-known theorem was adopted (Table 2) [10]. Continuous data were analyzed by a 2-way mixed, consistency single-measures ICC model and interpreted with the Cicchetti paradigm. All calculations were performed in IBM SPSS (v20.0).

Table 1
Interpretation of the intraclass correlation according to Cicchetti.

Intraclass correlation value	Interpretation
0–0.39	Poor
0.40–0.59	Fair
0.60–0.74	Good
0.75–1.0	Excellent

Table 2
Interpretation of the kappa value according to Landis and Koch.

Kappa value	Interpretation
0	None
0.01–0.20	Slight
0.21–0.40	Fair
0.41–0.60	Moderate
0.61–0.80	Substantial
0.81–0.99	Almost perfect
1.00	Perfect

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