Detection of the change in characteristics of self-grooming by the neural network in the latent period of the Rat Kainate Epilepsy model

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1. Introduction

Epilepsy is a chronic neurological disorder in which spontaneous seizures repeatedly occur. Human temporal lobe epilepsy (hTLE) is accompanied by severe motor seizures [1], which occur acutely immediately after brain injury and insult [2]. Subsequently, there is a seizure-free period during which the brain neuronal network is reorganized [3,4]; this period is known as the latent period. After this period, repetitive seizures occur during the chronic period [3,4], during which behavioural changes can be observed [5,6]. We assumed that behavioural changes may also be seen from the latent period. As human behaviour is complicated, we first studied the behavioural changes in a rodent model of epilepsy. Kainate (KA), a glutamate receptor agonist, induces epilepsy in rats [7]. The development of this model was similar to that of hTLE [7] and the model also has a latent period. Thus, we adopted the rat KA model to record behavioural changes during the latent period [7].

Maia et al. [8] reported that rats exhibited less anxiety-like behaviour during the chronic period in a KA rat model. We previously reported that KA administered rats exhibited less anxiety behaviour in the latent period [9]. The rats had longer locomotor distance with the faster running speed in the novel field and often entered the open arms in the elevated plus maze (EPM) task, while naïve rats usually do not [9]. In addition, the number of self-grooming bouts (episodes) significantly increased during the latent period [9]. Thus, for the first time, we report that behavioural changes can be observed in the latent period of the rat KA model. 

Self-grooming is an innate and hygienic behaviour of rats, which consists of specific and highly stereotyped patterns of sequential actions [10]. Self-grooming bouts consisted of the following phases: (0) no grooming, (1) paw licking, (2) nose/face/head grooming, (3) body grooming, (4) leg grooming, and (5) tail/genital grooming [11]. The serial transition structure of a chain tends to be repetitive and consistent in terms of order (e.g. 1–2, 2–3, 3–1). Self-grooming involves a basic chain of 0–1–2–3–4–5–0. The chain consists of six correct transitions (CT), e.g. 0–1, 1–2, 2–3, 3–4, 4–5, and 5–0. Self-grooming bouts are sometimes interspersed and stopped during the basic chain CT and comprise flexibly ordered mixtures of other motor behaviours, such as strokes, licks, or scratches. The chain includes incorrect transitions (e.g. 3–2, 3–0, 4–1) instead of CT. The ratios of correct and incorrect transitions in self-grooming bouts are different in rodent models of neuropsychiatric disorders [12]. In our previous study [9], we did not analyse self-grooming bouts in detail. Hence, in the present study, we examined the changes in the features of self-grooming bouts in the latent period of the rat epilepsy model to determine whether we could detect changes in the latent period of the rat model using these features.

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2. Materials and methods

The experiments were conducted in accordance with the Guide for the Care and Use of Laboratory Animals at the Graduate School of Life Science and Systems Engineering of the Kyushu Institute of Technology (Sei#2021-003).

2.1. Animals and treatments

Twenty-six male Wistar rats aged 3 weeks were provided by Japan SLC, Inc. The rats were kept in home cages at room temperature (23 ± 1°C), humidity (50% ± 5%), and controlled lighting (12-h light/12-h dark cycle). Food and water were provided ad libitum. The rats were handled for 7–9 days, at which point they were aged 4–5 weeks and used in the experiments. The rats were randomly divided into two groups: KA and control (n = 13 rats per group). In the KA group, KA was injected using the small-amount frequency method [7]. After anaesthesia with 3.5% isoflurane, the rats were injected intraperitoneally with 0.05% KA (10 ml/kg) every hour for a maximum of 3 h. The rats experienced motor seizures until the third injection. Seizures were scored according to the modified Racine score [7,13]. Class III–V motor seizures were considered. In class III, rats displayed forelimb clonus with lordotic posture; in class IV, rats were reared with concomitant forelimb clonus; and in class V, rats fell over in addition to a class IV seizure. The rats in the control group received the same anaesthesia as the KA group, and the same quantity of 0.9% saline (10 ml/kg) was injected intraperitoneally. On the day when rats were injected with KA, they had motor seizures, which paused within a day after the injection. However, 30–102 days after the injection, four to five of the KA group rats had seizures above Class III. Hence, we assumed that 8–10 days after the injection was the latent period and observed self-grooming at that time.

2.2. Analysis of self-grooming bouts

To evaluate the change in the self-grooming bouts in the latent period of the KA group, the rats were moved from the home cage to a novel transparent recording open field (50 × 50 × 35 cm) between 11:00 and 12:00 am. After acclimation for 20 min, we recorded self-grooming in the field for 40 min using a video camera (HC-V480MS, Panasonic Co., Japan). Self-grooming bouts included highly stereotyped patterns, as previously described. We measured three parameters of self-grooming bouts, as follows: (i) number, (ii) duration, and (iii) percentage of CT%. The duration was defined as the total time spent self-grooming divided by its number [14]. The self-grooming bouts include the following phases: no grooming (0), paw licking (1), nose/face/head grooming (2), body grooming (3), leg grooming (4), and tail/genital grooming (5) [11]. The bout was not terminated if at least one pause (< 6 s) was interspersed within the transitions of phases, whereas grooming bouts were terminated by interruptions longer than 6 s. The total transitions in self-grooming included CT and incorrect transitions. CT includes progressive transitions: 0–1, 1–2, 2–3, 3–4, 4–5, and 5–0 (each number represents the number of phases described previously). Incorrect transitions are chaotic and are characterized by skipping (e.g. 0–5 and 1–5) or reversed (e.g. 3–2, 4–1, and 5–2) and incorrectly initiated (e.g. 0–4 and 0–5) [10]. CT% is the ratio of the total transition numbers of the CT to the total transitions. The probability of transition from one phase to another was calculated for each rat. The overall patterns of the phase transitions can be calculated from the number of transition matrices. Table 1 shows an example of the transition matrices.

2.3. Detection of the change in self-grooming at the latent period of the rat KA model using a neural network

We used a three-layered neural network (NN) to detect whether the rat was in the latent period of KA epilepsy using self-grooming parameters. We provided several parameters of self-grooming bouts to the NN as the input. The NN had one to three units in the input layer depending on the dimension of the input data, 100 units in the intermediate layer, and two units in the output layer. The output indicated whether the input parameters were in the latent period. The number of units in each layer was adjusted to the optimum value. When the number of units decreased in the intermediate layer, the accuracy rate was lower; thus, 100 units were allocated to each layer. Each unit has an activation function for the rectified linear unit function. The loss function was a categorical cross-entropy function. The Adams function was used for the optimization. The NN was trained for 500 epochs in a trial, with a total of 20 trials. Seventy percent of the total parameters in both the KA and control groups were used for training the NN, among which, 30% were used for testing and evaluation. We calculated the accuracy rate for each trial, and the mean accuracy rate was calculated for the 20 trials. We used three parameters of self-grooming as input data, including the number, duration, and CT% in the latent period as input data of NN. The transition probabilities in CT were also used as input data. In some experiments, only one or two input parameters were selected from the three and given to the NN.
Table 1. Typical example of the difference in the transition probabilities of self-grooming bouts between before and after the injection in KA and control group rats.

| Stage | Control group | KA group |
|-------|---------------|----------|
| Before | After | Before | After |
| 0     | 1   | 0     | 1   |
| 1     | 0   | 0     | 0   |
| 2     | 0   | 0     | 0   |
| 3     | 0   | 0     | 0   |
| 4     | 0   | 0     | 0   |
| 5     | 0   | 0     | 0   |

The differences in the transition probabilities between before and after injection in a rat are shown in the bottom row. The top and middle tables indicate the probabilities before and after the injection in a rat. The tables at the left column indicate the probabilities of a control group rat and the tables at the right column indicate the probabilities of a KA group rat. The phase numbers at the row indicate the transition start phase and those at the column indicate the goal phase. Red cells indicate the transition probabilities of CT, and blue cells indicate incorrect transitions.

2.4. Statistical analysis

Statistical analyses were performed using R x64 3.5.0 (R Corp.). The significance level was set at 0.05. Data are expressed as mean ± standard deviation (SD).

3. Results

3.1. Modification of self-grooming bouts in the latent period

In the latent period, the KA group facilitated self-grooming bouts. The number of bouts was significantly increased in the latent period compared to that before injection (Figure 1; "p = 0.002, Wilcoxon signed-rank test [WSRT] with Bonferroni correction [BC]). The number of self-grooming bouts in the KA group was significantly larger than that in the control group during the latent period (Figure 1; "p = 0.002, Wilcoxon rank test [WRT] with BC). The duration of grooming was significantly longer than before injection in the KA group (Figure 2; "p = 0.009, WSRT with BC). The duration of the latent period of the KA group was significantly longer than that of the control group (Figure 2; "p = 0.01, WRT with BC).

The CT% in the latent period in the KA group was significantly higher than that before injection (Figure 3; "p = 13.3, 10^{-4}, WSRT with BC). The CT% in the KA group was significantly larger than that in the control group during the latent period (Figure 3; "p = 0.001, WRT with BC). We calculated the transition probabilities between the two phases among the five phases of the self-grooming bouts before and after injection in the KA and control groups, as shown in Table 1. The differences in transition probability of 1−2 in CT between before and after the injection were 6.7 in the control group, and 13.3 in KA group (Table 1). The difference of 1−2 in CT before and after the injection in the KA group was significantly larger than that in
Figure 2. Duration of self-grooming bouts in the latent period. *p < 0.05 and **p < 0.01.

Figure 3. CT% of self-grooming bouts in the latent period. **p < 0.01 and ***p < 0.001.

Figure 4. Difference in transition probabilities between before and after injection in the KA and control groups. Left and right bars in each transition indicate the transition probabilities in control and KA groups, respectively. *p < 0.05.

The control group (Figure 4; *p = 0.02, Kruskal–Wallis test [KWT], and Tukey’s honest significant difference [HSD] with BC as a post-hoc test).

3.2. Detection for the change in self-grooming bouts in the latent period in the KA group using the NN

Three parameters of self-grooming bouts (number, duration, and CT% in the latent period) were used as input data for NN training. The NN was trained using these data (Figure 5), and accuracy rates of 94% and 87.5% were obtained for training and testing, respectively (Figure 5). We studied the parameters that had the highest accuracy rate among the number, duration, CT% of self-grooming, and combinations. The accuracy rate using the input data including CT% was higher compared to that of the other parameters (Figure 6; KWT; ***p = 7.7 × 10^-5, and Tukey HSD test with BC as a post-hoc test; *p = 0.04 in N versus D, *p = 0.02 in D versus CT%, *p = 0.02 in D versus [N, CT%], *p = 0.01 in D versus [N, D, CT%]). Thus, the results suggest that CT% is a detectable feature of self-grooming during the latent period of the rat KA epilepsy model.

We then studied which transitions in CT were detectable during the latent period. Figures 7 and 8 show the transition probabilities of 1–2, 3–4, and 4–5, and that the combinations are predictive for the latent period of the KA rat epilepsy model. The mean accuracy rates for 1–2, 3–4, and 4–5 were 70.0, 71.9, and 71.8, respectively, with a significantly higher accuracy rate than that of 0–1 (Figure 7). The mean accuracy rates for 1–2–3, 3–4–5, and 4–5–0 were 71.8, 70.6, and 72.5, respectively (Figure 8).
4. Discussion

4.1. Facilitation of self-grooming bouts in the latent period of the KA epilepsy model

The KA group rats exhibited increases in the number, duration, and CT% of self-grooming bouts in the latent period (Figures 1–3). Systemic KA administration in rats induces acute seizures and decreased in the number of neurons in the hippocampus immediately after administration [15]. Hippocampal neurons project to the basal ganglia [16], and the basal ganglia neurons are also lost during the latent period [17]. The basal ganglia is related to motor control; thus, the basal ganglia is related to the CT control of self-grooming. When the output of basal ganglia neurons is suppressed, motor behaviour is facilitated. In this study, during the latent period, the output of basal ganglia neurons may be decreased due to the loss of basal ganglia neurons, and in the results, the KA group rats facilitated self-grooming. The reason why the KA group rats had significantly changed the transition probabilities of 1–2 in the latent period is unknown (Figure 4). Our previous study indicated that the locomotor distance increased during the latent period [9]. When striatal neurons are suppressed, the distance increases [18]; hence, the loss of striatum neurons by KA application leads to a change in self-grooming bouts as well as locomotion behaviour in the latent period. For the first time, we report that self-grooming can be increased during the latent period in a rat model of epilepsy.

4.2. Change in the CT of self-grooming bouts in the latent period of KA Group rats

CT% in the latent period was increased (Figure 3). Neurons in the ventromedial striatum of the basal ganglia are related to each phase of CT, while neurons in the dorsolateral striatum are related to CT transitions [19]. Serial transitions in CT are not made when the dorsolateral striatum is lesioned [20]. These results suggest that the KA epilepsy rat model undergoes a change in dorsolateral and ventromedial striatal neurons. In the future, we will attempt to identify the neuronal mechanisms that relate to changes in the latent period.

4.3. Detection of the change in self-grooming in the latent period

The accuracy rates of CT% and the combination including CT% were higher than those of the NN (Figure 6). The accuracy rates of the transition probabilities of 1–2, 3–4, and 4–5, and the combination including the transitions were the highest using the NN (Figures 7 and 8). Thus, we can detect self-grooming changes during the latent period using the NN with approximately 70% accuracy.

4.4. Human stereotypy may correspond to rodent self-grooming

Self-grooming in rodents can be used as a model for normal or pathological human grooming behaviour. Excessive self-grooming is a feature of some forms of mental disorders, such as obsessive-compulsive disorder (OCD) [21,22], and related illnesses, such as body dysmorphic disorder, excoriation (compulsive skin-picking), and trichotillomania (compulsive hair-pulling). OCD spectrum disorders are characterized by the excessive repetition of behavioural actions [23]. In this study, self-grooming was facilitated and its number increased in the latent period of the KA group rats (Figure 1).

In the case of human epilepsy, non-grooming stereotypes can be observed [6,24]. Human stereotypy is defined as repetitive behaviours involving abnormal or excessive repetition of a behavioural action in the same way over time [25], and represents the most common pathologically complex motor behaviour [6]. The striatum is involved in the generation of stereotypes [26]. Self-grooming is a model of complex, repetitive, self-directed, and sequentially patterned behaviours [12]. We assumed that the CT of rodent self-grooming corresponds to human stereotypes. The CT of self-grooming
was often terminated in the way of CT in control group rats, whereas complete CT was facilitated in KA group rats. Human stereotypy can also be interrupted and paused by other actions in healthy humans, whereas patients with hTLE perform stereotypy completely [6,24]. The change in stereotypy may be a sign of the epileptic latent period; therefore, we may be able to detect the change using some sensor devices in the future.

5. Conclusions

We examined changes in self-grooming bouts during the latent period in a rat model of KA epilepsy. The number, duration, and CT% of self-grooming bouts were facilitated during this period. The transition probability of 1–2 was higher than that of the other transitions. The latent period of KA group rats was detected with an accuracy rate of approximately 80% using the self-grooming parameters of CT% and 70% using transition probabilities of 1–2, 3–4, and 4–5 in CT. Thus, these results suggest that the CT% and some transitions in CT are the detectable features of self-grooming bouts change for the latent period of a rat KA epilepsy model.

Acknowledgements

We would like to thank Editage (www.editage.com) for English language editing.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work was supported by JSPS KAKENHI [grant number 16H06534 and 20K20838].

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