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Research paper

Spontaneous neural activity in the primary visual cortex of retinal degenerated rats

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HIGHLIGHTS

- Investigated the spontaneous neural activities in the primary visual cortex of RD model.
- Neurons in the primary visual cortex of RD model were hyperactive comparing to control group.
- Complexity of ISI sequence of spontaneous activities in the primary visual cortex of RD model decreased.

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ABSTRACT

Retinal degeneration (RD) models have been widely used to study retinal degenerative diseases for a long time. The biological and electrophysiological presentations of changes in the retina during degeneration progress have been well investigated; thus, the present study is aimed at investigating the electrophysiological effects of RD in the primary visual cortex. We extracellularly recorded the spontaneous neural activities in the primary visual cortex of RD rats. The firing rate, interspike interval (ISI) and Lempel-Ziv (LZ) complexity of spontaneous neural activities were subsequently analyzed. When compared to the control group, it was found that the neurons in primary visual cortex of the RD model fired more frequently. In addition, there was a decrease in LZ complexity of spontaneous neural firing in the RD model. These results suggest that the progress of RD may not only affect the retina itself but also the primary visual cortex, which may result in an unbalanced inhibition-excitation system as well as the decreased arising rate of new patterns of spontaneous activities.

1. Introduction

Retinal degeneration is the deterioration of the retina with the consequent death of retinal cells, especially photoreceptors, which results in progressive impairment or total loss of visual function. In order to better understand this disease, various animal models have been produced, such as mouse models *rd1*, *rd2*, as well as rat models RCS, P23H, and S334ter. Since mutations in the rhodopsin (*Rho*) gene are very common in RD and contribute to the majority of known genetic forms of autosomal dominant (ad) retinitis pigmentosa (RP), substantial efforts have been made to develop models that express rhodopsin mutation [1]. Among all the rat models of RD, P23H and S334ter express rhodopsin aggregation defect and rhodopsin inactivation defect, respectively [1]. Specifically, S334ter rhodopsin transgenic rats, as a model of several rhodopsin truncation mutations in human RP patients, express rhodopsin gene with an early termination codon at residue 334, resulting in the expression of a rhodopsin protein without 15C-terminal amino acids that are involved in rhodopsin trafficking to the photoreceptor outer segments and in the inactivation of rhodopsin protein after light absorption [2,3]. Although there are several lines of S334ter rat with different rates of photoreceptor degeneration, there is a similar course of photoreceptor degeneration followed by secondary modifications [4,5].

Among the research on RD models, many efforts have been made in the scope of electrophysiology, as electrical activities play an essential role in early development of the nervous system, as well

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as at later stages in refinement of connections [6]. In particular, spontaneous activities have been considered to be important for the refinement of neural projections and maintenance of topographic maps (neuroanatomy) in the brain [7,8]. There has been substantial evidence showing that altering the pattern of spontaneous activities disrupts this refinement [9].

The spontaneous activities, therefore, have caught major attention in the research community. Notably, an increase in spontaneous hyperactivity of retinal ganglion cells (RGCs) occurs during the progression of retinal degeneration [10–13], and this spontaneous hyperactivity is sustained well into adulthood for weeks after photoreceptors have disappeared [11]. It has been reported that an increase in glutamate concentration and a decrease in photoreceptors partially contributes to the increase in the spontaneous firing rate of ganglion cells in RD models [10,12].

The research on RD models, however, represents a bias towards physiology and electrophysiology of the retina, or cortical organization in the macular degeneration subject by analyzing the fMRI data [14–16]. The data on spontaneous activities in the visual cortex of RD models is still insufficient. Therefore, in the present study, the spontaneous activities, particularly the spiking in primary visual cortex of RD rats, have been investigated, and we expect to know the changes in inherent neural activities in the primary visual cortex of RD model in terms of electrophysiological properties.

2. Material and methods

2.1. Animals and surgical procedures

The animals were obtained from Animal Center of Chinese University of Hong Kong, and housed in animal facilities at City University of Hong Kong. All experiments were conducted in accordance with protocols approved by Animal Research Ethics Sub-Committee in City University of Hong Kong and Department of Health, HKSAR. Long Evans and S334ter rats (P80–P90, n = 5 for each group), were used as the control (wild-type, WT) and RD groups, respectively.

For surgery, the procedures were similar to those in previous work [17]. The animal was anesthetized with intraperitoneal injection of Ketamine- Xylazine combination (Ketamine: 70 mg/kg, Xylazine: 7 mg/kg) initially, then isoflurane (2%) was applied during the recording.

After craniotomy, a bone screw was fixed in the skull and a glass-pipette tungsten electrode was placed on the surface of primary visual cortex (AP: −6 ∼ −9 mm; ML: 2 ∼ 4 mm; DV: −0.1 ∼ −1 mm), as the ground and recording electrode, respectively. The exposed cortical surface was covered with 2% agarose to avoid drying and reduce body oscillation caused by breath, then a shield was used to attenuate the environmental interference. The animal was maintained at 38 °C by a warm pad, and an ocular solution was regularly applied to keep the eyes moist during the surgery and recording.

2.2. Recording

The A-M Systems 3600 (A-M Systems, US) and CED Micro 1401-3 (Cambridge Electronic Design, UK) were used as the amplifier and data acquisition system, respectively. By using a micromanipulator, the recording electrode was lowered into the brain. The reading was recorded as zero reference when the electrode just passed through the cortical surface.

As spikes appeared, the electrode was suspended frol m advancing and was allowed to settle for approximately 10 min before recording. The room was kept dark during the extracellular recording of spontaneous activities. The signal was filtered from 300 to 5 kHz and sampled at 25 kHz. The electrodes were placed in various penetrations and depths in order to reach different layers of the primary visual cortex. In total, three to four locations per penetration, and two to three penetrations were recorded for each animal. For every location, the recording lasted over 5 min. At the end of recording, the animal was euthanized by overdose of Dorminal (300 mg/kg).

2.3. Histology

After the recording, eyeballs of both WT and RD animals were enucleated and fixed in 10% formalin in 0.1 M phosphate buffer (pH 7.4) at 4 °C for overnight. Samples were then dehydrated with a graded series of ethanol and xylene and subsequently embedded in paraffin wax. Retinal sections (5 μm) with pupil-optic nerve position were stained with hematoxylin and eosin (H&E). Retinal sections were imaged with a light microscrope to assess the status of different layers of retina.

2.4. Data analysis

Spike sorting was initially conducted to distinguish between single units. A MATLAB (R2012b, MathWorks, US) package named Wave_Clus [18] was used for this purpose, and the units were classified based on the features of their waveforms.

After spike sorting, the firing rate of spontaneous activities was calculated. To compare the distribution of firing rates of different groups, we first performed the Shapiro-Wilk test to evaluate the normality of the data distribution. If the data satisfied a normal distribution, a *t*-test was conducted; otherwise, the Mann-Whitney test was used.

The ISI was subsequently obtained. For every single unit, the mean and LZ complexity of ISI sequence were determined for comparing the ISI distribution and complexity of all the units from both WT and RD groups. Specifically, for calculating the LZ complexity, an algorithm based on Abraham Lempel and Jacob Ziv's work was applied [19]. The statistical tests were as same as above.

3. Results

3.1. Degeneration in RD retina

The H/E staining of both WT and RD rat retinas showed that the thickness of degenerated retinas of RD rats at P90 is less than that of the age matched control group (Fig. 1). Specifically, the outer plexiform layer (OPL), outer nuclear layer (ONL) as well as photoreceptor inner and outer segments (IS/OS) had almost disappeared. This validated the condition of retinal degeneration in RD model, which also agrees with previous studies [2,4,5,20]. Moreover, it has been reported that the inner nuclear layer (INL), inner plexiform layer (IPL) and ganglion cell layer (GCL) appear intact even at the age of P226 [21]. In the present study, however, we found that these layers in the RD retinas were also notably atrophied at the age of P90 when compared to the WT retinas.

3.2. Spontaneous firing rate

In the present study, 96 single units from 5 WT rats and 89 single units from 5 RD rats were isolated through spike sorting. Then, the representative waveforms of spontaneous activities were plotted on top of the averaged curve (Fig. 2).

We have investigated the spontaneous activity of single neurons in both WT and RD groups. First, we determined the firing rates for each unit in both groups. In the WT group, the firing rates displayed a range from 0.11 Hz to 17.49 Hz with the mean ± SD of 3.04 ± 3.77 Hz. In the RD group, the firing rates showed a range from 0.11 Hz to 24.52 Hz with the mean ± SD of 4.05 ± 4.17 Hz.