The Classification of EEG Waves Recorded from the Telencephalon in Carp

Hiromasa YOSHIKAWA*, Soichi NAKAMURA**,
Haruki MATSUI*,*1 and Yoshinari ISHIDA**,*2

* *Interdisciplinary Research Institute of Environmental Sciences, Nishi-iru, Hichihon-matsu, Itsutsuji-dori, Kamigyo-ku, Kyoto 602-8317, Japan*

** *Department of Fisheries, Kinki University, Naka-machi, Nara 631-8505, Japan*

*1 *Present address: 1-26 Yoshida-hon-machi, Sakyo-ku, Kyoto 606-8317, Japan*

*2 *Present address: Japan Marine Science College, 1-7-3 Sangen-ya-higashi, Taisho-ku, Osaka 551-0001, Japan*

Synopsis

In this study we deal with an objective method of identifying the boundary between two contiguous EEG frequency bands and the classification of EEG waves recorded from the telencephalon in carp. First, a paired t-test was carried out at 0.1 Hz intervals between the EEG-power obtained by fast Fourier transform at defined two epochs, and the t-files, which consisted of t-values at the ordinate and frequencies at the abscissa were made. Next, the t-values were smoothed by using a moving average, and frequencies with a t-value of zero were determined. From the zero-crossing frequencies, telencephalic EEGs were classified into five frequency bands, 1.5-3.9, 4.0-6.9, 7.0-10.9, 11.0-16.9, and 17.0-30.0 Hz.

Introduction

There have been several reports on EEGs in fish [1-9]. In these studies, one or two dominant frequency ranges identified by visual inspection were usually employed for the analysis of EEGs. The dominant frequency range is variable with the fish species and brain region: 4-6 Hz for the telencephalon, 8-13 and 14-32 Hz for the optic tectum, 14-32 Hz for the medulla oblongata in cod, *Gadus callarias* [1]; 4-8 and 9-14 Hz for the telencephalon, 7-14 and 18-24 Hz for the optic tectum, 25-35 and 120-180 Hz for the cerebellum in goldfish, *Carassius auratus* [2]. In goldfish, the other classification of dominant frequency ranges was proposed [6]: 6-8 and 16-24 Hz for the telencephalon, 6-9 and 16-24 Hz for the optic tectum, 6-9 and 15-20 Hz for the cerebellum. The discrepancy between those studies in goldfish may result in the difference of experimental conditions, as pointed out by the latter author, such as EEG recording in a bipolar or monopolar lead and with or without anesthetic. However, it seems to be due in part to the subjective identification of the frequency ranges by visual inspection. Using fast Fourier transform or linear prediction analysis, tectal EEGs in carp, *Cyprinus carpio*, were classified into three contiguous frequency bands, 4-7, 8-13, and 14-25 Hz [8]. However, its ground for determination of a beginning or end of the frequency bands was obscure.

In fish, the telencephalon has been shown to be associated with olfaction, arousal responses, and reproductive behavior [10-15], but the physiological significance of telencephalic EEGs is still unclear. Here, we report an objective method of identifying the boundary between two contiguous frequency bands and the classification of telencephalic EEGs in carp.

Materials and Methods

We have examined the effect of acute change in environmental conditions on telencephalic EEGs recorded in a bipolar lead in carp, each weighing about 500g, which were immobilized with d-tubocurarine chloride under artificial ventilation [16-19]. The EEG data, which had been stored on magnetic tape, in carp subjected to MS222 (tricaine methanesulfonate) [16] or CO_2 anesthesia [17], hypoxia [18], hyperthermia [19], or hypothermia [19] and in the respective control carp were used in the present study. The experimental protocol and breeding condition were described in the reports cited above.

First, 1-min EEG signals without artifacts were analyzed every 10 seconds by fast Fourier transform and an average power spectrum was calculated at seven particular periods in each experiment: at 0, 7, 11, and 30 min during 30 min anesthesia and at 17, 30, and 60 min after anesthesia in case of MS222 or CO_2 anesthesia; at 15 min intervals during 60 min acute environmental change and subsequent 30 min periods in case of hypoxia, hyperthermia, or hypothermia. The EEG data at these periods, such as the raw EEG waves and average power spectra, have been shown in the reports cited above.

Next, a paired t-test with ten carp was carried out at 0.1 Hz intervals between the EEG power at two epochs in every combination with seven particular periods in each above-mentioned experiment. Then, t-values obtained by the paired t-test were shown as a t-file where the positive t-value denotes a decrease in the EEG power and the negative t-value denotes an increase, with the t-value at a level of $p<0.05$ regarded as significant.

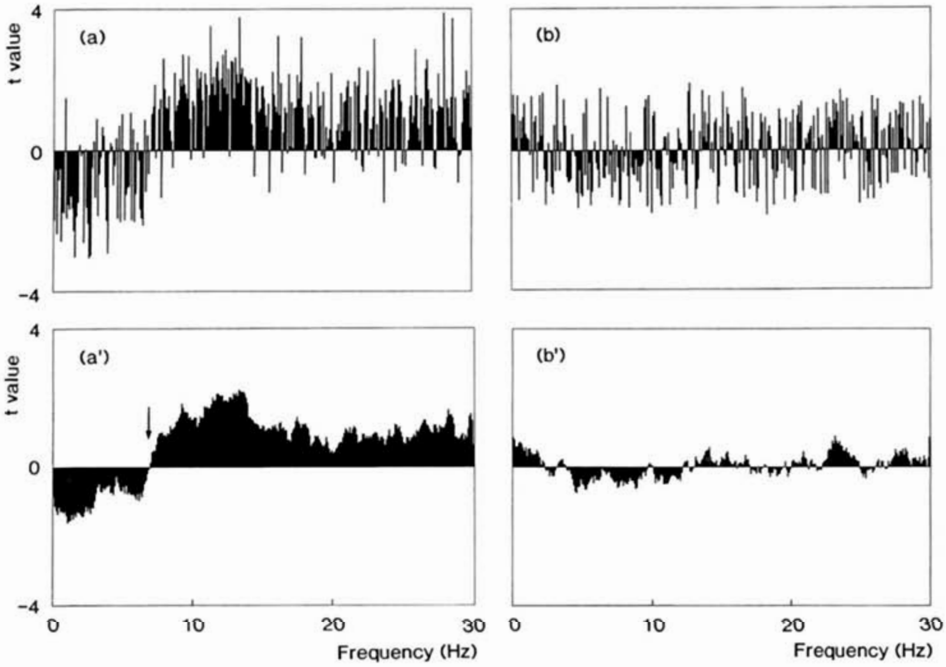

Fig. 1. Statistical analysis by paired t-test between the EEG power at different two periods. In this and the following figures, using each group of ten carp, t-values were obtained at 0.1 Hz intervals ranging from 0 to 30 Hz. (a): a t-file showing comparison between the EEG power at 17 and 30 min after 30 min MS222 anesthesia, (b): a t-file showing comparison between the EEG power in the control carp at two periods similar to in (a), (a') and (b'): t-files smoothed by moving average of 7 points. As for the number of serial t-values used for the moving average, see the text. An arrow indicates a boundary between two frequency bands.

In some t-files, a defined frequency range showed a pattern as a whole different from other frequency ranges. For example, as shown in Fig. 1a, most of the frequencies below about 7 Hz showed negative t-values, whereas most of those above 7 Hz showed positive t-values. We attempted to apply a smoothing technique by using the moving average to the t-file. The result is shown in Fig. 1a' which is smoothed using the moving average of 7 serial t-values. For reference, an original t-file and a smoothed t-file in the control carp are shown in Fig. 1b and Fig. 1b', respectively. No marked boundary was recognized in the smoothed control t-file, showing small negative or positive t-values. These results strongly suggest that about 7 Hz is a boundary between the two contiguous frequency bands. Therefore, to detect the other boundaries and determine an accurate frequency corresponding to the boundary, we made the 105 smoothed t-files obtained by using the moving average of 7 to 15 serial t-values in every combination with seven particular periods in each experiment. The number of serial t-values used for the moving average was determined so that all t-values of a defined frequency range in either side of a boundary showed a sign, positive or negative, different from those of the other frequency range. We also made the smoothed t-files in the control carp obtained in the same way as in the carp subjected to the above-mentioned treatment.

Results and Discussion

Five boundaries, about 1.5 Hz (Fig. 2a), about 4 Hz (Fig. 2a,b), about 7 Hz (Fig. 2c), about 11 Hz (Fig. 2c,d), and about 17 Hz (Fig. 2d), could be detected. It should be noted that all of the five boundaries were generally detected in the smoothed t-files for each above-mentioned experiment, and that the ambiguous boundaries, such as a boundary (Fig. 3a) like a bottom between two frequency ranges with the same sign of negative or positive and a boundary (Fig. 3b) without the t-value above the significance level of $p < 0.05$ in both sides in an original t-file, were not taken into account, although ambiguous boundaries were usually recognized at similar frequencies. In addition, no marked boundary could be found in the control t-files. The data concerning the five boundary frequencies are summarized in Table 1.

The analysis of EEGs has usually been performed by comparing two epochs, before external stimulus and a subsequent period during or after the stimulus. However, in this study, few boundaries could be detected by this routine method. In most cases, a defined frequency range demonstrated an evident increase or decrease in the EEG power, while other frequency ranges remained unchanged. These showed a small negative or positive t-value in the t-file, or else all frequencies ranging from 0 to 30 Hz showed t-values with the same sign, being either negative or positive. Most of the boundaries were found by comparing between two defined periods during environmental change, or between two defined periods during and after the change. In this context, we considered that the t-files to be used for the present purpose were not restricted to those by comparison between the EEG power before the exposure and at a subsequent period, and that any two periods could be used. Even if two contiguous frequency bands demonstrated a similar pattern, such as an increase in the EEG power during exposure and recovery to the initial level at a subsequent period, a boundary between the contiguous frequency bands might be detected under the following conditions: (1) after both bands increased in the EEG power at the first measurement during exposure, one frequency band showed a further increase while the other band conversely showed a decrease, although continuing to show a higher power than that before exposure when the second measurement was taken during subsequent exposure, or (2) before both bands were restored to the initial level after exposure, one band transiently increased while the other band gradually decreased without a transient increase. This is probably because the

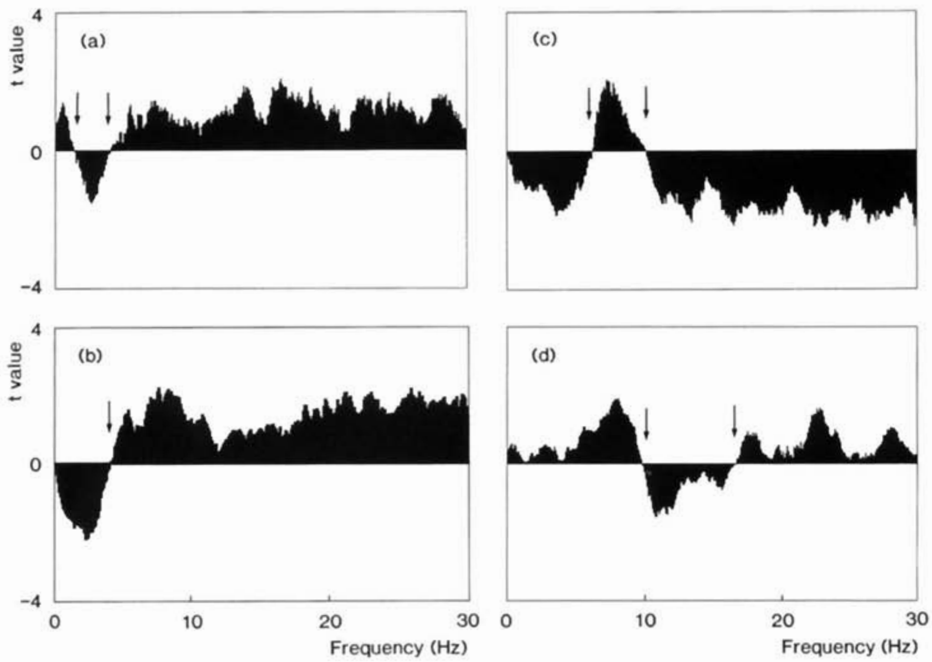

Fig. 2. Smoothed t-files showing comparison between the EEG power at 30 and 60 min during 60 min hypoxia (a), comparison between the EEG power at 7 min during 30 min CO_2 anesthesia and at 30 min after anesthesia (b), comparison between the EEG power at 15 min during 60 min hypothermia and at 15 min after hypothermia (c), comparison between the EEG power at 15 min during 60 min hypothermia and at 15 min after hypothermia (d). Each arrow indicates a boundary between two frequency bands. The numbers of serial t-values used for the moving average were 11, 13, 13, and 11, respectively.

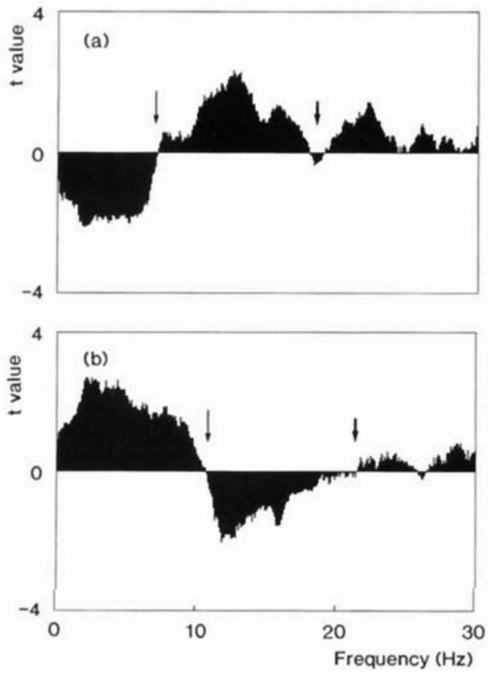

Fig. 3. Smoothed t-files showing comparison between the EEG power at 30 min during MS222 anesthesia and at 17 min after anesthesia (a) and comparison between the EEG power at 17 and 60 min after MS222 anesthesia (b). Each fine arrow indicates a boundary between two frequency bands, and each bold arrow indicates an ambiguous boundary which was not regarded as a boundary. These two smoothed t-files were obtained by the moving average of 11 serial t-values.

responses, whether an increase or decrease in EEG power compared with that before external stimuli, of five frequency bands to environmental changes used in this study were similar in quality, but different in time course, for example, recovery to the initial level with or without a transient change after external stimuli. Our method probably detects differences in the time course of responsiveness of two contiguous frequency bands.

Generally, it is difficult to determine a minimum or maximum frequency of EEGs. Also, in fish EEGs as in human EEGs, the DC component or steady potentials usually below 2 Hz are known to change in response to external stimuli [20-23]. Although about 1.5 Hz was regarded as a boundary in this study, it seems that the greater part of the component below 1.5 Hz resulted from artifacts produced by body movement or changing pressure of electrodes on the brain surface and was superimposed on an original frequency band of 0-3.9 Hz. Tectal EEG waves above 4 Hz were employed for identification of the frequency bands in restrained carp because of artifacts by respiratory movement of about 3 Hz or below [8]. The artifacts of relatively low frequency in this study were probably due to the use of a muscle relaxant. The maximum frequency could not be determined in this study, but an EEG component above 30 Hz was scarce in telencephalic EEGs in carp.

Therefore, we propose the five frequency bands, 1.5-3.9, 4.0-6.9, 7.0-10.9, 11.0-16.9, and 17.0-30.0 Hz, in telencephalic EEGs in carp. As shown in Table 1, each boundary has some variations in frequency, indicating little significance of a decimal fraction in frequency.

Table 1. The classification of telencephalic EEGs in carp.

Boundary frequency* in Hz			Band	
(mean±SD)	range	n**	No.	Frequency range in Hz
1.5±0.3	1.2−1.9	16	1	1.5−3.9
4.1±0.4	3.3−4.8	13	2	4.0−6.9
6.9±0.3	6.4−7.3	14	3	7.0−10.9
10.8±0.8	9.8−11.9	8	4	11.0−16.9
17.2±0.5	16.4−17.8	5	5	17.0−30.0

* a minimum frequency within a frequency band: when there are two bands of 0-a Hz and b-30 Hz in both sides of a boundary, b Hz is shown as a boundary frequency because a boundary itself has no frequency.

** the number of each boundary found in the 105 smoothed t-files.

The identification of EEG frequency bands by visual inspection of the wave form or power spectrum is difficult because beginnings and ends of the frequency band can only obscurely be determined. Using the present method, five frequency bands of telencephalic EEGs in carp were detected objectively, but a great deal of data were required to detect each frequency band. A more convenient method would be necessary to classify the frequency bands of EEGs in other fish species or brain regions.

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コイの終脳脳波の分類

吉川弘正*, 中村聡一**, 松井春樹*,*1, 石田義成**,*2

* (財)環境科学総合研究所

** 近畿大学農学部水産学科

要　　約

本研究では、隣接する脳波の周波数帯域の境界部を客観的に同定する方法とコイ終脳脳波の分類について報告する。まず、任意の２時期間において、高速フーリエ変換により求めた脳波のパワー値を0.1Hz毎にpaired t-testにかけ、横軸に周波数そして縦軸にt値を示したt-fileを作成した。次に、t値を移動平均によりスムージングし、t値がt-file上で零点と交差する周波数を求めた。零点交差周波数から、コイの終脳脳波を５種類の周波数帯域(1.5-3.9、4.0-6.9、7.0-10.9、11.0-16.9、17.0-30.0 Hz）に分類した。

*2　日本海洋科学専門学校