Power Spectral Analysis of Olfactory Bulbar Responses to Amino Acids for Carp

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Synopsis

The olfactory bulbar electroencephalograms of carp resulting from stimulation with a 10^{-2} mol solution of 19 L-α-amino acids were analyzed by fast Fourier transform. Each pH of the test solution for 14 neutral amino acids was adjusted to the isoelectric point, p*I*, ranging from 5.1 to 6.3, and each pH for 3 basic and 2 acidic amino acids was adjusted to the p*I* (10.8 for L-arginine, 9.7 for L-lysine, 7.6 for L-histidine, 2.8 for L-aspartic acid, 3.2 for L-glutamic acid) or to a mean value of the p*K*s for the α-amino and α-carboxyl groups of each amino acid, which was termed p*I'* in this study, ranging from 5.5 to 5.9. The power spectra for neutral amino acids resembled each other, although the total power varied with the amino acid. Their responsive frequency ranges were 7 to 15 Hz. The basic amino acids showed higher peak frequencies and greater total power at the p*I*s than at the p*I'*s. The acidic amino acids showed lower peak frequencies and smaller total power at the p*I*s than at the p*I'*s. The responsive frequency ranges for the basic amino acids were 3 to 15 Hz independently of the pH, whereas those for the acidic amino acids were 3 to 15 Hz at the p*I*s and 3 to 20 Hz or higher at the p*I'*s. The olfactory bulbar activities were classified into three components, 3-7 Hz, 7-15 Hz, and 15-20 Hz or higher, and a 7-15 Hz component was common to the amino acids examined.

Introduction

Amino acids are potent olfactory stimuli in fish[1]. Olfactory responses to amino acids have been examined electrophysiologically at various levels of the fish olfactory pathway[2-13]. The responses recorded from the olfactory bulb have also been studied, since a stable response can be measured for a long period according to OHNO *et al.*[14]. In most studies of fish olfactory bulbar electroencephalograms (EEGs), the amplitude of the summated response using an electronic integrator was employed as an index of the olfactory response[15-21]. The summated response may be useful for the evaluation of the stimulatory intensity or threshold level, but less effective for the discrimination of stimuli, since the amplitude of the summated response varies with the concentration of stimuli.

Spectral analysis has been attempted in order to establish a frequency-coding mechanism in the olfactory bulb of fish[22-26]. In the rainbow trout, *Oncorhynchus mykiss*[22], the bulbar response to food extracts showed a multi-peak spectrum, unlike the response to amino acids with a single-peak spectrum. Some pesticides had spectral profiles greatly different from those for amino acids in the carp,

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Cyprinus carpio[26]. These findings suggest that a certain chemical or chemical group has its own peak frequency or specific spectrum. A marked difference in the peak frequency or overall spectral profile between amino acids has not been observed, but this may be due to the fact that few amino acids have been examined by spectral analysis.

In some electrophysiological studies, the pH of the amino acid solution was roughly adjusted to neutral, usually by the addition of NaOH or HCl. Fish smell amino acids in ambient water the pH of which is usually in the vicinity of neutrality. In other studies, the amino acids were just dissolved in water or sea water without pH adjustment, probably with the viewpoint that pH is one of the chemical features of the amino acids. Little attention has been paid to the influence of the HCl or NaOH added for pH adjustment or to the pH change itself.

HARA[15] proposed an amino acid-specific receptor site which involves two charged subsites capable of interacting with ionized α-amino and α-carboxyl groups, on the basis of the experimental result that amino acids induced maximal bulbar responses near their isoelectric points (pIs) in rainbow trout. Judging from his data in rainbow trout, however, L-aspartic acid showed a maximal response near a mean value of the pKs of the α-amino and α-carboxyl groups rather than the pI which is a mean value of the pKs of the α-carboxyl and β-carboxyl groups. Moreover, the response to basic amino acids was not illustrated in the HARA's report. The maximal responses to basic or acidic amino acids may be obtained at their mean values of the pKs of the α-amino and α-carboxyl groups rather than at their pIs.

In the present study, the olfactory bulbar EEGs on stimulation with 19 L-α-amino acids were analyzed by fast Fourier transform in carp, with special reference to the influence of pH on the spectral profile of basic and acidic amino acids. In addition, the bulbar responses to water of various pHs were examined.

Materials and Methods

The experiments were carried out on 30 carp, *Cyprinus carpio*, of either sex, each weighing about 400g (410±40g, mean±SD). Carp were obtained from a commercial supplier. They were kept for 6 months at 23±1℃ under a 14-h light/10-h dark cycle in indoor plastic tanks. They were fed a commercial diet for carp.

First, each carp was immobilized by an intramuscular injection with d-tubocurarine chloride (5mg/kg weight) in aerated water. The carp was then wrapped in a moist cloth, fixed to an experimental holder, and artificially ventilated through the mouth with dechlorinated tap water. The flow rate and temperature of the water for artificial ventilation were held constant at 1 L/min and 23℃, respectively.

To expose the olfactory bulb, the skull roof was opened and mesenchymal tissues were removed. Two Ag-AgCl electrodes with ball tips (0.2mm in diameter) for recording bulbar EEGs were placed on the dorsal surface of the olfactory bulb and on the skull, respectively. An Ag-AgCl disc electrode was attached as an earth electrode to the body surface. Bulbar EEGs were amplified with an AC amplifier (AVB-10, Nihon Kohden) through a bandpass filter of 1.5-30Hz, monitored by an oscilloscope (VC10, Nihon Kohden), and stored on magnetic tape by a data recorder (MR10, Teac) for later analysis.

Before the beginning of the experiment, distilled water was perfused into the carp's nasal cavity through a glass capillary. Using a remote solenoid valve with three flow ways (P/N225, NResearch), stimulation to the naris was performed by replacement of the distilled water with test solution. After the 5-s stimulation with test solution, the olfactory epithelium was immediately rinsed with distilled water. The interstimulus intervals were at least 5 min. The flow rate and temperature of rinsing water and test solution were held constant at 0.2 mL/s and 23℃, respectively. The experimental setup used in the present study was essentially the same as that described by ISHIDA *et al.*[26].

Three kinds of experiments were carried out on groups of ten carp each. In the first group, the effect of pH on bulbar activities was examined with water of pH3 to pH10 made by adding 0.1 N of NaOH or HCl to distilled water. In the second group, the bulbar responses to stimulation with 10^{-2} mol of 14 neutral L-α-amino acids were examined, each pH being adjusted to the respective isoelectric

point, pI, ranging from 5.1 to 6.3. In the third group, the bulbar responses to stimulation with 10^{-2} mol of 3 basic and 2 acidic L-α-amino acids were examined, each pH being adjusted to the pI (10.8 for L-arginine, 9.7 for L-lysine, 7.6 for L-histidine, 2.8 for L-aspartic acid, 3.2 for L-glutamic acid) or a mean value of the pKs for the α-amino and α-carboxyl groups of each amino acid (ranging from 5.5 to 5.9) which is termed pI' in the present study. The data for the pI and pK values of the amino acids were taken from the CRC Handbook of Chemistry and Physics[27]. The pH adjustment for the amino acid solution was also achieved by adding 0.1 N of NaOH or HCl, but the pHs of all amino acids (10^{-2} mol) dissolved in distilled water were similar to their respective pIs. L-threonine and L-cysteine were obtained from Kokusan Chemicals and Gibco Laboratories, respectively. The other amino acids were obtained from Wako Pure Chemicals.

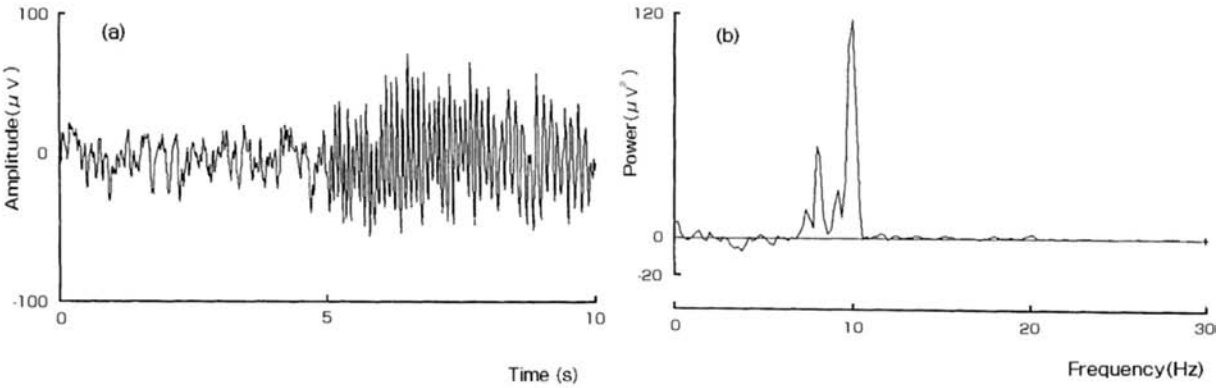

Fig. 1. The power spectral analysis used in the present study, with the carp bulbar response to 10^{-2} mol of L-threonine as a representative result. (a): Approximately 10 s of data consisting of spontaneous waves just before the stimulation and evoked waves during the stimulation; (b): A differential power spectrum obtained by subtracting each power corresponding to the frequency range from 0 to 30 Hz for the spontaneous waves from that for the evoked ones. From the differential power spectrum for each carp, the peak frequency (approximately 10 Hz in this case), total power (0 to 30 Hz), and overall spectral profile including the presence of a sub-peak (approximately 8 Hz in this case) were examined. A responsive frequency range was estimated from the mean power spectrum made by averaging the differential power spectra of ten carp.

The data analysis was performed using a signal processor (7T18A, Nihon-denki Sanei), as follows. The bulbar EEGs, which had been stored on magnetic tape by using a switching signal of the remote solenoid valve as a trigger, were digitally converted with a sampling clock of 9.766 ms, and divided into spontaneous waves of 512 points just before the stimulation and evoked waves of 512 points during the stimulation. An initial point in the evoked waves was determined by visual inspection (Fig. 1a). Approximately 10-s data consisting of 1024 points were analyzed every 512 points by fast Fourier transform (frequency resolution of approximately 0.2 Hz, window of Hanning), and a differential power spectrum was obtained by subtracting each power corresponding to the frequency range from 0 to 30 Hz at 0.2 Hz intervals for the spontaneous waves from that for the evoked waves (Fig. 1b).

The evaluation of statistically significant differences was made using the Wilcoxon rank sum test. All data are presented as mean $\pm$ SD. The level of significance was defined as $p < 0.05$.

Results

First, the effect of pH change on the olfactory bulbar activity was examined between pH 10 and pH 3. Figure 2 shows the mean power spectra of bulbar responses to water of pH 10(a), 9(b), 7(c), 6(d), 5(e), and 3(f). When individually examined, these power spectra were characterized with multiple peaks in a wide range of frequencies. There was no prominent peak, such as the peak of approximately

10 Hz for L-threonine shown in Fig. 1b. Judging from the mean power spectra in Fig. 2(a, f), there seemed to be two responsive frequency ranges, 3 to 7Hz and 7 to 15 Hz. Table 1 summarizes the mean peak frequency and mean total power. It was evident that water of pH 10 or pH 3 induced a bulbar response, compared with the total power (-0.8± 15.4μV^2) for distilled water, the mean total power being 90.1±31.1μV^2 for pH 10 and 62.7±71.4μV^2 for pH 3. No marked difference between the mean power spectra for pH 10 and pH 3 was recognized. The mean peak frequencies for pH 10 and pH 3 were 5.4±2.7 and 6.1±2.3 Hz, respectively.

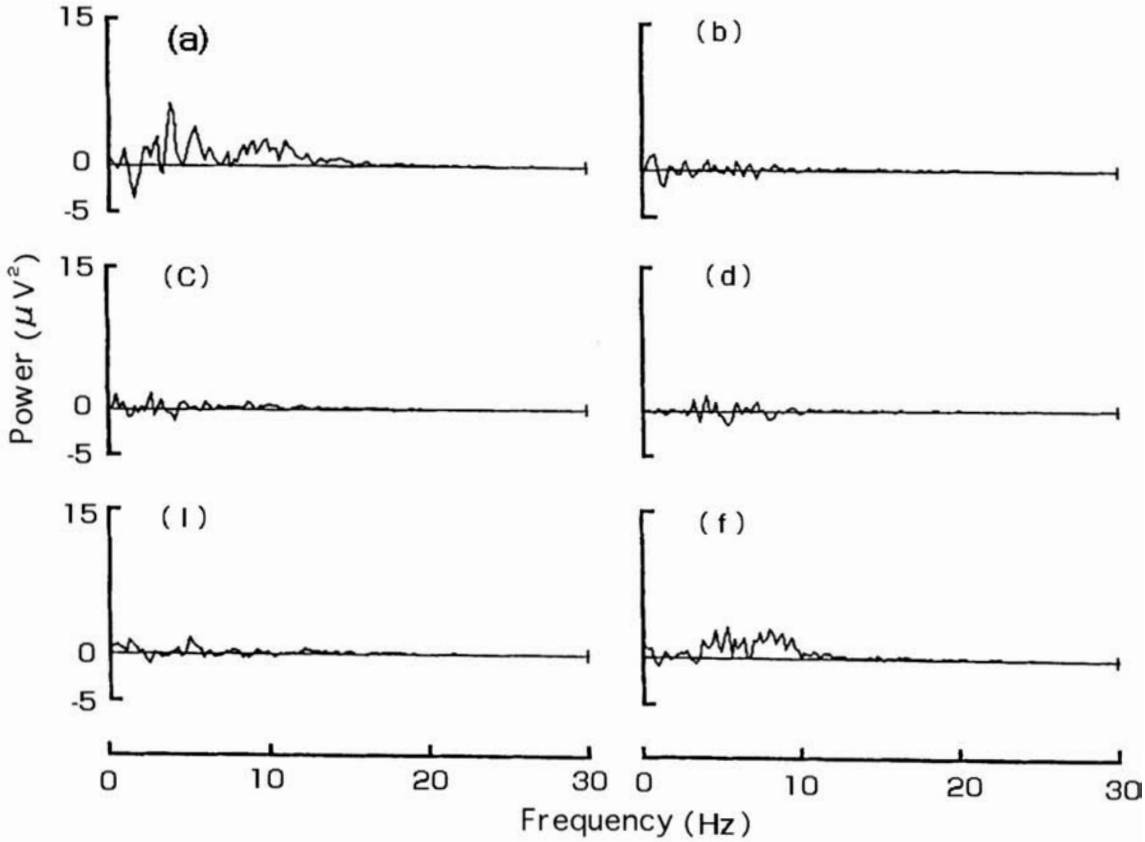

Fig. 2. The mean power spectra of carp bulbar responses (n=10) to stimulation with water of pH 10(a), 9(b), 7(c), 6(d), 5(e), and 3(f).

Table 1. The peak frequency and total power of carp bulbar responses to stimulation with water of various pH levels.

pH	Peak frequency (Hz) mean±SD	Total power (μV^2) mean±SD
10	5.4±2.7	90.1±31.1
9	———	6.8±17.5
7	———	13.6±17.5
6	———	−0.8±15.4
5	———	11.3±23.7
3	6.1±2.3	62.7±71.4

Figure 3 shows the mean power spectra obtained by stimulation with 10^{-2} mol of 14 neutral L-α-amino acids, in order of the total power. Each pH was adjusted to the pI, ranging from 5.1 to 6.3. As described above, a small amount of NaOH or HCl was required for the pH adjustment. As a whole, the mean power spectra for neutral amino acids resembled each other. Their peak frequencies were approximately 10 Hz, and their responsive frequency ranges were roughly 7 to 15 Hz. The responsive frequency range or the peak of approximately 10 Hz was somewhat ambiguous in the mean power spectra for neutral amino acids with relatively small total power, such as L-isoleucine, L-phenylalanine, L-tryptophan, and L-proline, but the individual power spectra for these amino acids were similar to those of the other neutral amino acids, when individually examined. Table 2 summarizes the mean peak frequency and mean total power in order of the total power. The mean peak frequencies ranged from 8.4 to 11.0 Hz, and the mean total power ranged from 58.7 to 283.3μV^2. The mean peak frequencies for L-tryptophan and L-proline were relatively low, but higher than those for water of pH 10 and pH 3.

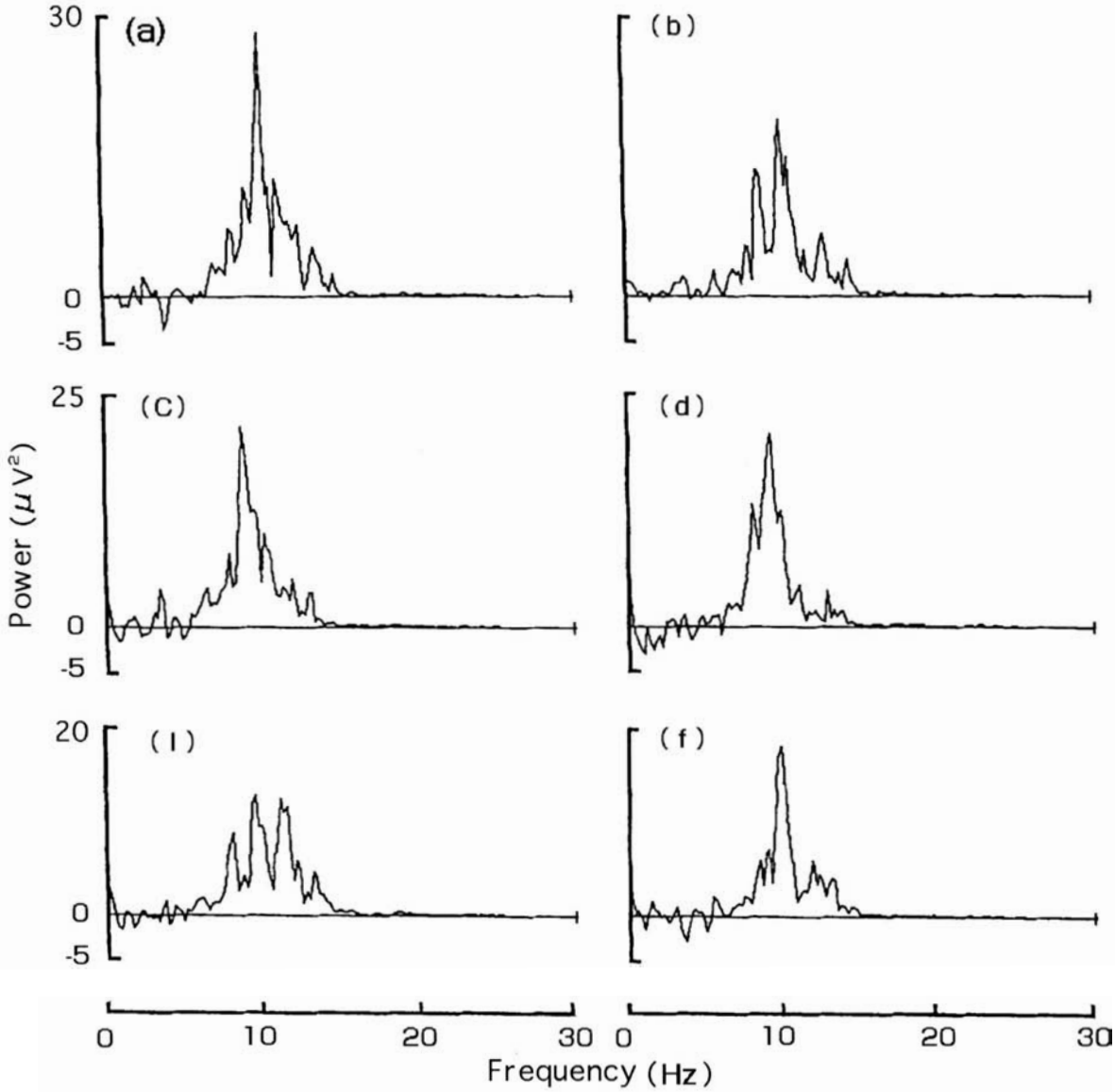

Fig. 3－1. The mean power spectra of carp bulbar responses (n＝10) to stimulation with 10^{-2} mol of the neutral amino acids, L-threonine (a), L-alanine (b), L-serine (c), L-cysteine (d), L-asparagine (e), L-glutamine (f).

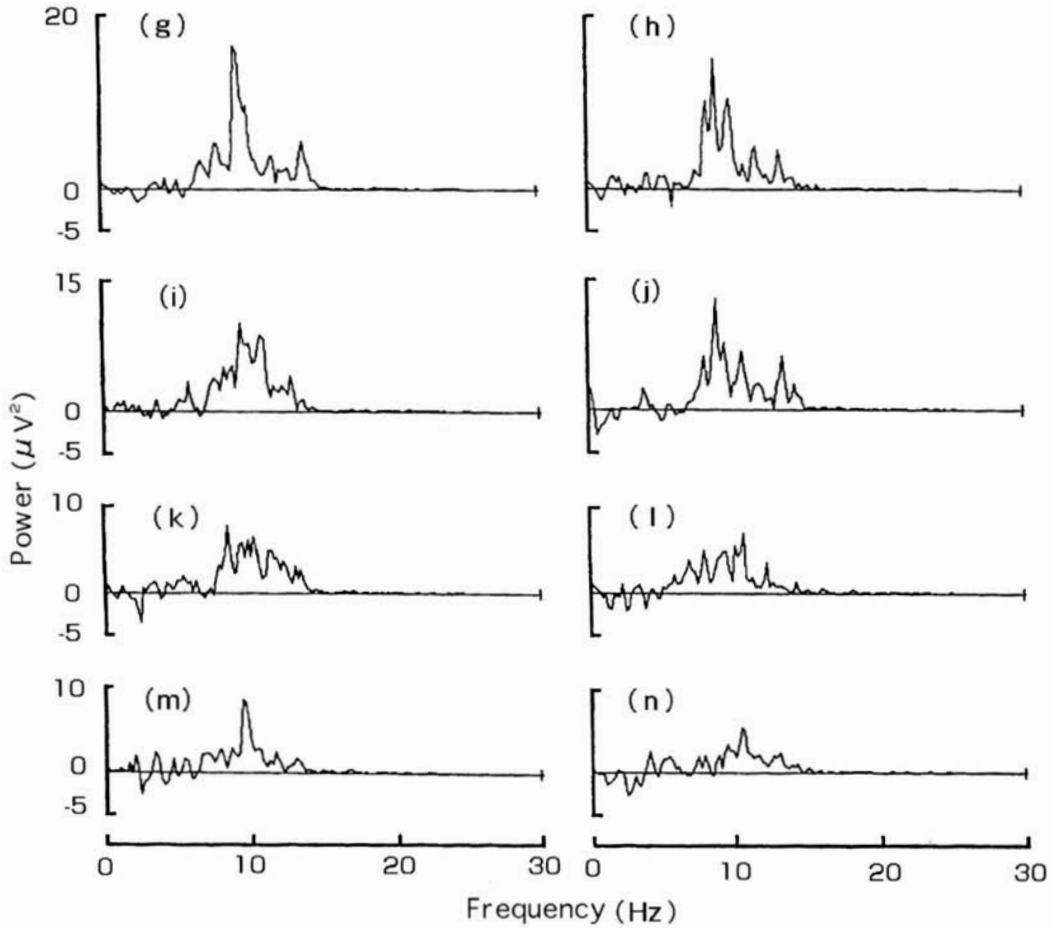

Fig. 3−2. The mean power spectra of carp bulbar responses (n=10) to stimulation with 10^{-2} mol of the neutral amino acids, L-methionine (g), L-leucine (h), glycine (i), L-valine (j), L-isoleucine (k), L-phenylalanine (l), L-tryptophan (m), and L-proline (n).

Table 2. The peak frequency and total power of carp bulbar responses to stimulation with 10^{-2} mol of 14 neutral amino acids listed in oider of the total power.

Amino acids[a]	Peak frequency (Hz) mean±SD	Total power (μV^2) mean±SD
L-threonine	10.6±1.3	283.3±181.9
L-alanine	11.0±2.1	261.6±141.3
L-serine	9.1±1.2	245.2±145.2
L-cysteine	9.4±0.9	218.5±177.8
L-asparagine	10.5±2.0	218.2± 97.7
L-glutamine	10.0±2.2	186.3±125.0
L-methionine	10.4±2.1	165.7± 91.0
L-leucine	9.8±1.8	159.9±104.6
glycine	9.7±1.7	159.4± 73.4
L-valine	10.1±1.6	134.0± 73.6
L-isoleucine	10.5±2.4	123.2± 72.5
L-phenylalanine	9.4±2.4	99.3± 57.1
L-tryptophan	8.7±3.0	82.6± 59.7
L-proline	8.4±3.0	58.7± 30.5
D. W.[b]	———	1.6± 13.9

[a] The pH of each amino acid was adjusted to the isoelectric point
[b] pH 5.9±0.1

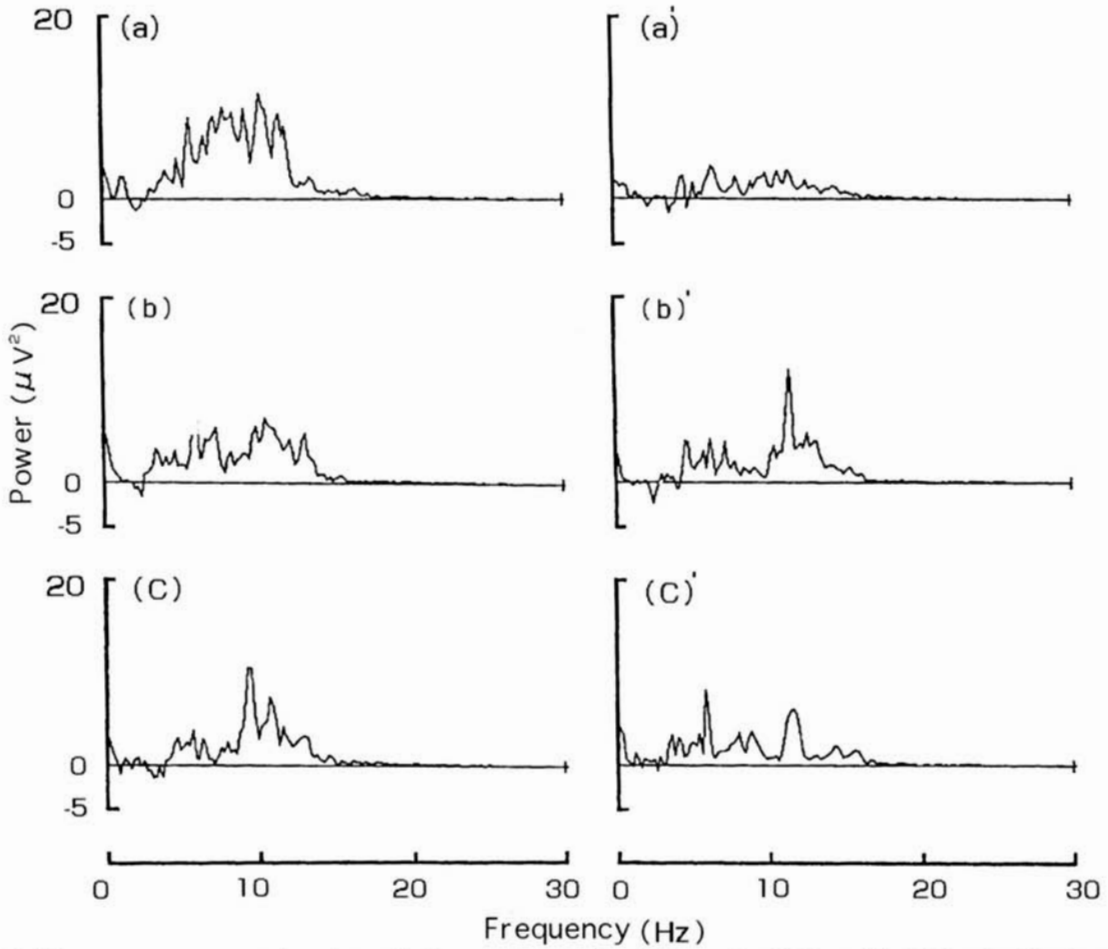

Fig. 4. The mean power spectra of carp bulbar responses (n=10) to stimulation with 10^{-2} mol of basic amino acids, L-arginine (a, a'), L-lysine (b, b'), L-histidine (c, c'). (a-c): The pHs were adjusted to the p*I*s, (a'-c'): The pHs were adjusted to the mean values of the p*K*s for α-amino group and α-carboxyl group of each amino acid.

Figure 4 shows the mean power spectra of the bulbar responses to stimulation with 10^{-2} mol of three basic L-α-amino acids, the pHs of which were adjusted to their p*I*s (a-c) or p*I*'s (a'-c'). Table 3 summarizes the mean peak frequency and mean total power of the responses to the basic and acidic amino acids. Independently of the pH (p*I* or p*I*'), the individual power spectra for the basic amino acids were characterized with multiple sub-peaks in a wide range of frequencies and a prominent peak. As a result of the multiple sub-peaks, the mean power spectra were rather broad in shape. The difference between the power spectra at the p*I*s and p*I*'s was evident, although the responsive frequency range, 3 to 15 Hz, was common. The basic amino acids tended to show higher peak frequencies and greater total power at the p*I*s than at the p*I*'s. Significant differences were recognized between the total power for L-arginine 315.3 $\pm$189.0μV^2 at the p*I* and 88.1$\pm$47.9μV^2 at the p*I*', and between the peak frequency for L-histidine 10.1$\pm$1.6 Hz at the p*I* and 8.3$\pm$3.0 Hz at the p*I*'.

Figure 5 shows the mean power spectra of the bulbar responses to stimulation with 10^{-2} mol of 2 acidic L-α-amino acids, the pHs of which were adjusted to the p*I*s (a, b) or p*I*'s (a', b'). As was the case for the basic amino acids, the individual power spectra had a prominent peak and multiple sub-peaks. The mean power spectra at the p*I*s and p*I*'s were more different than was the case for the basic amino acids. The acidic amino acids showed lower peak frequencies and smaller total power at the p*I*s than at the p*I*'s (Table 3). These differences were all significant. In addition, it should be noted that the responsive ranges were roughly 3- 15 Hz at the p*I*s and 3-20 Hz or higher at the p*I*'s.

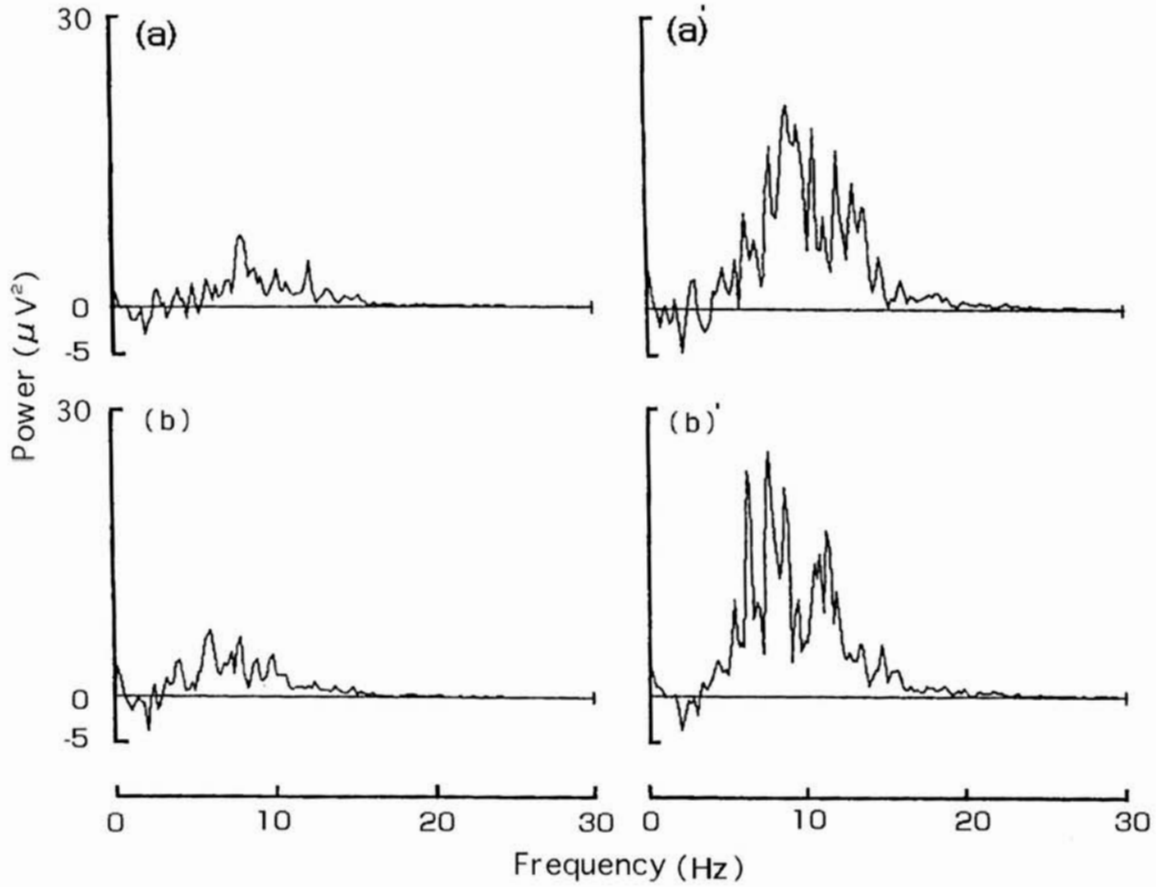

Fig. 5. The mean power spectra of carp bulbar responses (n=10) to stimulation with 10^{-2} mol of acidic amino acids, L-glutamic acid (a, a'), L-aspartic acid (b, b'). (a, b): The pHs were adjusted to the p*I*s, (a', b'): The pHs were adjusted to the mean values of the p*K*s for α-amino group and α-carboxyl group of each amino acid.

Table 3. The peak ferquency and total power of carp bulbar responses to stimulation with 10^{-2} mol of 3 basic and 2 acidic amino acids.

Amino acids[a]	Peak frequency (Hz) mean±SD		Total power (μV^2) mean±SD	
	p*I*	p*I'*	p*I*	p*I'*
L-arginine	9.0±2.2	7.6±3.0	315.3±189.0	88.1± 47.9**
L-lysine	8.8±2.7	7.6±3.0	221.9±156.9	162.4±115.9
L-histidine	10.1±1.6	8.3±3.0*	160.3± 71.8	151.2±144.6
L-glutamic acid	6.2±2.0	8.3±2.8*	126.9±125.4	517.4±357.8**
L-aspartic acid	7.3±2.0	9.4±1.9**	109.5± 86.5	497.8±392.0*

[a] Each pH was adjusted to the isoelectric point, p*I*, 10.8 for L-arginine, 9.7 for L-lysine, 7.5 for L-histidine, 3.2 for L-glutamic acid, 2.8 for L-aspartic acid, or a mean value of the p*K*s of the α-amino and α-carboxyl groups of each amino acid, which is termed p*I'*, 5.6 for L-arginine, 5.6 for L-lysine, 5.4 for L-histidine, 5.9 for L-glutamic acid, 5.8 for L-aspartic acid.

*$p<0.05$, **$p<0.01$

Discussion

The olfaction of carp seemed to be less sensitive to pH change, because an evident response was recognized in the pH change of 3 or 4 units in the present study. Although there have been few studies on fish olfactory responses to pH change, species differences are known to exist. In rainbow trout, the bulbar response to L-serine was inhibited at pH values below 3.5[15]. However, such inhibition by low pH did not occur in yellowtail, *Seriola quinqueradiata*[21], as well as the carp examined in this study. The response to water of low pH was also recorded from the olfactory tract in carp[4].

In studies of the concentration-dependent response or threshold level of amino acids, the olfactory responses to amino acids were examined at a wide range of concentrations, from 10^{-8} to 10^{-1} mol[2, 3, 5, 8, 10-14, 16, 17, 19-21]. The responses to higher concentrations of amino acids were suspected to encompass the response to the osmolarity, pH, or acidic or basic ions added for pH adjustment, but there have been few studies on factors other than the amino acid itself.

In the present study, to obtain the distinctive power spectra of carp olfactory bulbar responses to amino acids, a relatively high concentration (10^{-2} mol) of amino acids was employed because some amino acids are known to produce a relatively small response in carp[3, 14]. According to HARA *et al.*[22] and ISHIDA[28], the peak frequency or overall profile of the power spectrum was known to be independent on the concentration of amino acids in rainbow trout and carp. These results suggest little or no influence of changes in osmolarity.

The involvement of three factors in the response to amino acids must be considered: H^+ or OH^-, Na^+ or Cl^- for pH adjustment, and the amino acid itself. It was found in the present study that the mean power spectra for basic or acidic water had two frequency ranges, 3 to 7 Hz and 7 to 15 Hz. It is difficult to assign the frequency ranges to these ions which do not exist alone. From the finding that the responsive frequency range for neutral amino acids was 7 to 15 Hz, H^+ or OH^- and/or Na^+ or Cl^- is thought to be responsible for the 3-7 Hz component. However, L-histidine also had a 3-7 Hz component at the p*I* (pH 7.6) and p*I'* (pH 5.5); No or just a small amount of NaOH or HCl was required for the pH adjustment. Assuming that the basic amino acids had the responsive frequency range of 3 to 15 Hz in common, the 3-7 Hz component for the basic amino acids would be dependent not on H^+ or OH^- and/or Na^+ or Cl^-, but on the basic amino acid itself. In the case of the acidic amino acids, the responsive frequency ranges were dependent on the pH: 3-15 Hz at the p*I*s and 3-20 Hz or higher at the p*I'*s. In addition, a component of 15 to 20 Hz or higher was specific for acidic amino acids at the p*I'*s. The factor responsible for the 3-7 Hz component of acidic amino acids is unknown; a relatively large amount of NaOH was required for the pH adjustment to the p*I'*s, and the low pH corresponding to the p*I*s was enough to induce a bulbar response.

In the spectral analyses done thus far[22-26], the power spectrum was individually obtained from bulbar activities consisting of the olfactory response and background activity. Consequently, the responsive frequency range, especially a component with smaller power, could not be identified. In the present study, the responsive frequency range could be determined by calculating the differential power spectrum. Judging from the responsive frequency range, the neutral, basic, and acidic amino acids can be discerned from each other, and a 7-15 Hz component was found to be common to the amino acids examined. In addition, the bulbar activities in carp may be classified into three components, 3-7 Hz, 7-15 Hz, and 15-20 Hz or higher.

The mean power spectra for the acidic amino acids, L-glutamic acid and L-aspartic acid, at the p*I*s (pH 3.2 and 2.8) closely resembled that for water of pH 3 regarding the overall spectral profile, mean peak frequency, and responsive frequency range. The total power for the acidic amino acids were somewhat greater than that for the pH 3 water: $126.9 \pm 125.4 \mu V^2$ for L-glutamic acid, $109.5 \pm 86.5 \mu V^2$ for L-aspartic acid, and $62.7 \pm 71.4 \mu V^2$ for the pH 3 water. Taking the response induced by the low pH into consideration, the total power for the acidic amino acids themselves were rather small, although their power ($517.4 \pm 357.8 \mu V^2$ for L-glutamic acid and $497.8 \pm 392.0 \mu V^2$ for L-aspartic acid) at the p*I'*s were the largest among the amino acids examined in the present study. The olfactory responses to amino acids are assumed to be affected

by the binding affinity to the specific receptors because of a change in the electrification of the amino acids by pH. A similar result was obtained in rainbow trout, whose bulbar responses to L-aspartic acid at pH 5 and pH 4 were approximately 170 and 50 % of that at pH 7.5, respectively[15].

There have been three studies including the present study on the olfactory responses to amino acids in carp[3, 14], but the rank order lists of the stimulatory effectiveness of amino acids are somewhat different from each other. This may be due to the difference in the level of the olfactory pathway (the olfactory bulb or olfactory tract), analytical method (summated response or spectral analysis), or pH of the amino acid solution used. L-threonine and L-serine, L-glutamic acid and L-aspartic acid, and L-phenylalanine and L-tyrosine are known to have a common receptor in carp, respectively[14]. Their power spectra were also similar in the present results of all parameters of the peak frequency, total power, responsive frequency range, and overall spectral profile, although the bulbar response to L-tyrosine was not examined in the present study.

Several studies suggested that different receptor types for amino acids exist in the olfactory cells[2, 5, 10, 11, 16]. These studies were usually carried out using amino acids with various substituted groups. In related to pH, an amino acid-specific receptor site which involves two charged subsites capable of interacting with ionized α-amino and α-carboxyl groups was proposed in rainbow trout[15]. In carp, the basic amino acids tended to show greater total power at the p*I*s than at the p*I*'s, and the acidic amino acids showed greater total power at the p*I*'s than at the p*I*s. These results do not completely agree with the above-mentioned hypothesis[15]. In addition, the charged β-carboxyl group has been shown to be unnecessary for the receptor response in channel catfish, *Ictalurus punctatus*[2], and American eel, *Anguilla anguilla rostrata*[5].

However, an influence of the difference in the electrification of the amino acid itself was suggested by our findings that the peak frequencies for L-histidine at the p*I* (pH 7.6) and p*I*' (pH 5.5) were significantly different, and a 15 to 20 Hz or higher component was specific for the acidic amino acids at the p*I*'s. These were probably independent of the H^+ or OH^- and Na^+ or Cl^-. In addition, the magnitude of the bulbar responses to L-serine changed two-fold at pH values between 5 and 6 in rainbow trout[15]. To clarify the relationship between the olfactory response and pH, further studies are necessary, especially on the response to pH change itself and acidic or basic ions for pH adjustment.

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アミノ酸に対するコイの嗅球応答の周波数解析

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要　約

19種類のL-α-アミノ酸の10^{-2}モル溶液で刺激した際のコイの嗅球脳波を高速フーリエ変換を用いて解析した。14種類の中性アミノ酸試験液のpHは各アミノ酸の等電点（pI，5.1～6.3）に調整し，3種類の塩基性アミノ酸および2種類の酸性アミノ酸の試験液のpHは各アミノ酸の等電点（L-アルギニンは10.8，L-リジンは9.7，L-ヒスチジンは7.6，L-アスパラギン酸は2.8，L-グルタミン酸は3.2）または各アミノ酸のα-アミノ基とα-カルボキシル基のpKの平均値（pI'と仮称，5.5～5.9）に調整した。中性アミノ酸のパワー・スペクトルはお互いに類似していたが，パワー値はアミノ酸により異なった。中性アミノ酸に対して反応する周波数帯域は7～15ヘルツであった。塩基性アミノ酸は，pI'よりpIの方がピーク周波数が高く，パワー値が大きかった。酸性アミノ酸は，pI'よりpIの方がピーク周波数が低く，パワー値が小さかった。塩基性アミノ酸の反応周波数帯域はpHに関係なく3～15ヘルツで，酸性アミノ酸の反応周波数帯域はpIで3～15ヘルツ，pI'で3～20ヘルツまたはそれ以上であった。嗅球脳波は3～7ヘルツ，7～15ヘルツ，15～20ヘルツまたはそれ以上の3成分に分類できた。また，7～15ヘルツの成分は本研究で調べたアミノ酸に共通していた。

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