Odor input generates ~1.5 Hz and ~3 Hz spectral peaks in the *Helix* pedal ganglion

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**Abstract**

In 1999 we reported that odorants evoke in the *Helix* pedal ganglion (PG) activity patterns which are largely odorant-specific and related to the nature of odor and its behavioral output. Notably, some activities (for example, ~1.5 and ~3 Hz), nonspecific to odorants, were consistently evoked in PG. The present contribution goes farther in a deeper survey of the intrinsic and odorant-evoked activities of PG with special weight on the nonspecific fluctuations. We address the following questions. (i) What are the features of the activities? (ii) Are they comparable to the activities found in the motor systems of the other invertebrates? (iii) To what functions can they be related? Three main frequency components represented by power peaks at ~1 Hz, 1–2 Hz and 2–8 Hz seem to feature the response activities of PG. (a) The aversive odorants induce odorant-specific patterns represented by peak power frequencies at ~1 Hz. (b) The oscillation at ~1 Hz, which exists intrinsically in the *Helix* PG, can be specifically enhanced by appetitive odors. Activities induced in the procerebrum (PC), the visceral ganglion (VG) and PG by appetitive odorants, such as ethanol and apple, peak at 1.3–2 Hz, whereas those induced by aversive ones, such as formic acid and onion at ~1 Hz. (c) The 2–8 Hz components always accompany the odorant-evoked activities of the PG either as the second or third strongest component, or in the form of conspicuous, long-lasting ~3 Hz oscillations. (d) The nonspecific odor-evoked 1–2 Hz and ~3 Hz activities, and the intrinsic ~1 Hz activity of the PG seem to be interrelated by a degree of mutual exclusion. We may therefore consider these activities as elementary, slow components that are involved in the processing of signals in this ganglion. It can be inferred from the findings in other invertebrates that the 1–3 Hz spontaneous discharge is strongly connected with motor activity that involves the feedback mechanism of the procerebro-cerebro-buccal or -procerebro-cerebro-pedal circuit. Our approach differs from most others reported so far in the following aspects: (i) use of gross steel electrodes for recording population activities; (ii) lengthy stimulation (10 min); (iii) long observation during and after stimulation; (iv) power spectral presentation of temporal evolution of activity patterns; (v) estimation of peak power frequency by Frequency–Amplitude Plot (FAP) (obtained from signals averaged in the frequency domain; a method based on systems theory).

**Theme:** Motor systems and sensorimotor integration

**Topic:** Invertebrate motor function

**Keywords:** Odorant-evoked oscillation; *Helix* pedal ganglion; Power spectra; Frequency–amplitude plot; Memory for controlling locomotion

1. **Introduction**

A change of spontaneous oscillation of the procerebral lobe, that is caused by odorant input, is linked to a phenomenon of olfactory information processing [14,15,18,19,21,25,26,36,38,39,43]. There is also an increasing number of accounts in the literature that relate dynamical alteration of activity pattern to information processing of the central pattern generator circuitry involving the pedal, cerebral, buccal and other ganglia [1,11–13,20,22,23,28,31,34,36,38–40]. It is hypothesized that a strong feedback mechanism exists among these centers. Recent morphological study [33] of the *Helix* CNS reveals that there are, in PC, subsets of cells that send neurites to
the PG and to the other parts of the ipsilateral as well as contralateral cerebral ganglion (CG).

Olfaction is one of the essential stimuli for the snail to recognize the environment and closely associated with locomotion behavior. For better understanding of this, it is important to study odorant-related activity patterns generated in the PG where locomotion is largely regulated.

We regard odorant-evoked ongoing field potentials (FPs) as population responses and analyze them relying on the descriptive efficiency of frequency analysis that is based on systems theory [2,3]. We have, for the first time, demonstrated that odorants can be discriminated by low-frequency components that have a degree of specificity for odorants or their classes [36,38,39]. According to our findings, the activities of <15 Hz, in particular, the <8 Hz components, are most strongly involved in processing of odor information in the PC. The peak power frequencies <2.5 Hz of responses seem to play decisive roles in discriminating odorants (e.g., -0.2 Hz for ammonia, ~0.4 Hz for formic acid, ~0.5 Hz for 2-pentanol, ~0.7 Hz for 2-butanol, ~1.3 Hz for ethanol, ~0.3 Hz for onion and ~1.1 Hz for apple).

In 1999 we showed that odorants evoke in the *Helix* PG activity patterns which are largely odorant-specific and related to the nature of odor and its behavioral output and that some unique, nonspecific components and/or oscillations <8 Hz are also evoked with certain regularity (for example, 1–2 Hz components and ~1.5 Hz oscillations; 2–8 Hz components and ~3 Hz oscillations) [38,39]. The present contribution goes farther in describing the features of the intrinsic and odorant-evoked activities of PG in more detail by additional analysis with a special weight on the nonspecific activities. In the present study, we addressed the following questions. (i) What are the features of these activities? (ii) Are they comparable to the activities found in the motor systems of the other invertebrates? (iii) To what functions can they be related?

2. Materials and methods

2.1. Experimental procedure

The details have been described in the previous report [36] and here we state only the outlines of the methods. We used the isolated preparation of the whole CNS with the intact sense organs and semimicroelectrodes (steel, 100 μm in diameter) for recording. Activity was recorded shortly before (<4 min; control), immediately after (<5 s), 4–5 min, 8–10 min, and 12–15 min, sometimes longer, after stimulus onset. The stimulus was normally removed at 8–10 min after stimulus onset. As odorants ethanol, 2-butanol, 2-pentanol, formic acid and ammonia with various dilutions and two natural odorants, onion and apple, were used.

For data acquisition and analysis, we used a software package specially developed by BrainData® (Lübeck, Germany). We took records of either 20 epochs (≈204.8 s) or 40 epochs (409.6 s) at a time with digitization at 200 Hz in samples of 2048 per epoch (one epoch = 10.24 s). The data of 40 epochs were then analyzed by power spectral plots of single epochs. The average frequency–amplitude plot (FAP) was determined for 20 consecutive epochs.

Fourier spectral analysis quantifies the amount of activity in frequency bands, but the information related to time evolution of frequency peaks is neglected. Therefore, in our study, visual assessments of the time records and power spectral sequence are important for the interpretation of the FAPs and the estimation of the peak power frequencies.

2.2. Frequency–Amplitude Plot (FAP); \( G(j\omega) \)

According to general systems theory, any linear system can fully be described either in the time domain or in the frequency domain. The TRFC-method (the Transient Response Frequency Characteristic method) is known, in systems theory, as a method for the analysis of linear systems. We apply this method as a first approach to the study of the brain, which is active and nonlinear like most biological systems [2]. Errors resulting from the length of measurements are assumed to be larger than the errors due to the systems linearization.

The type of nonlinearities in the brain, however, may cause some distortions in the response pattern. But, these distortions are reflected in the frequency–amplitude characteristics only as some minor peaks and insignificant double peakings [2].

The frequency–amplitude characteristics of a fluctuating system can be described by the following equation. We call the resulting plot, which depicts each frequency and its amount of activity as relative amplitude, the Frequency–Amplitude Plot (FAP).

\[
G(j\omega) = \int x(t) \exp(-j2\pi ft) dt = \text{FAP}
\]

where \( x(t) \) = the time history of the pattern to be analyzed, \( G(j\omega) \) = complex representation of the Fourier-transformed time series, \( \omega = 2\pi f \), the angular frequency, \( f \) = frequency of the input signal, and \( j = \sqrt{-1} \), the imaginary unit.

FAP gives the transfer function which would convert white noise into the Fourier spectrum. The presumed advantage is that it discards absolute power for relative power to an arbitrary scale (0 dB). FAP attempts to emphasize the relative heights of peaks in each sample by emphasizing their absolute power by normalizing to the lowest frequency passed by our filter. We do not claim this value has a unique comparability across samples of activity: the zero of the ordinate is arbitrary and fluctuates, relative to all other frequencies, depending on the amount
of very slow potentials shift (0.1 Hz and, with some attenuation, lower frequencies) at that time.

We used a spectral averaging method (averaging in the frequency domain): We first computed frequency characteristic of each epoch (10.24 s) and then the average of all 20 epochs (= 204.8 s) as average FAP. To determine the peak power frequency, we superimposed the average FAP of the response on that of the control (the average of 204.8 s activity recorded within a few minutes before presentation of odor) and by eye estimated the frequency, at which the power increase was largest. When the increase was strong, the peak power frequency matched that of the ongoing activity during odor exposure. Each individual FAP was normalized according to an arbitrarily defined voltage, although 0 dB was not always the same in μV (a typical value was 0 dB = 3 μV).

2.3. Statistical evaluation

To give confidence in the results, we calculated 95% confidence limits according to Student [45] for the average values of the peak power frequencies.

3. Results

Because we believe some of the power or FAP peaks, especially in the very low frequencies, are due to irregular or transient slow events that cannot be reasonably called rhythmic, we have reserved the term oscillation for fairly regularly repeated events visible in the raw time series and used more general, inclusive terms, such as activities or fluctuations to embrace either these or irregular fluctuations or both.

3.1. Spontaneous activities

To describe the intrinsic activity of the isolated PG, we surveyed spontaneous activities (40 epochs = 409.6 s) of several preparations before applying any odor stimulation. In this manner we avoided any ‘carry-over effect’ of an odorant-evoked event.

One important feature was that the intrinsic activity of PG without odor stimulation was dominated by low-frequency fluctuations at <1 Hz (the strongest power at 0.31 ± 0.05 Hz and the next at 0.63 ± 0.09 Hz, 95% confidence limits, the values estimated from FAPs of the averages calculated for the first and second 20 epochs separately).

Another property of the spontaneous discharge was the ~1 to 1.5 Hz activity. The examples from three preparations are shown in Fig. 1. A majority (9 out of 12 preparations) exhibited either in the form of constant spikes (Fig. 1, II and second day, I) and/or short rhythmic bursts lasting 10–60 s (Fig. 1, I and III) [38,39]. However, the power of this ~1 Hz activity was normally

Fig. 1. Spontaneous ~1 Hz activities of the Helix pedal ganglion. Examples from three different preparations I, II and III are shown. Time records of a few consecutive epochs starting at the bottom. Although the unstimulated preparation (without intentionally administered odors) can show a variety of activities, as illustrated, typically they maintain the same kind of activity for many minutes or hours, so that a prestimulus recording is a suitable control for recorded responses to odorants. Preparation I: an example where spikes and occasional ~1.3 Hz oscillation were superimposed on the dominating low-frequency (<0.5 Hz) fluctuations. Preparation II: a case where spike activity at ca. 1.1 Hz was clearly visible. Preparation III: a rare case where rhythmical oscillation at 1.1–2 Hz lasted at least for 1 min. Preparation I, second day: Note that the activity of the <0.5 Hz component became much lower than the first day. Calibration: 70 μV for Preparation I and 25 μV for the rest.
much lower compared with that of the frequency range <1 Hz. The other two showed strong slow wave fluctuations <1 Hz. The FAPs depicted a broad, dominant component peaking at 0.3–0.4 Hz and another much weaker one at 0.7–1.4 Hz. One had only a weak activity <1 Hz peaking at 0.3 Hz and no spikes. The spiky activities (Fig. 1, Prep. II and Prep. I, second day) remained stable, but the type with wave oscillations (Fig. 1, Prep. I and III) went through the stages where the wave burst slowly decreased the frequency and amplitude finally becoming only spike activities. This cycle repeated every 1.5 to 2 min.

Nevertheless, this phenomenon was unique to PG and rendered a primary difference from PC and VG, since neither PC nor VG showed such ~1 Hz spontaneous discharge in Helix under the present experimental conditions.

3.2. Variation of odorant-induced activities

Oscillatory responses of PG to odorants can be classified into three major groups according to the pattern of power increase [38,39]. To elucidate any interrelations among these groups, we calculated the average peak power frequencies for each stimulant and presented in Table 1 the numerical values together with degree of aversiveness (the parameter as distance, in cm, to the odor source at the tentacle withdrawal reflex; cf. Ref. [36]). In Fig. 2, we plotted the frequencies in the order of aversiveness. We named the responses according to the order of their incidence among many trials and preparations: Most common response pattern = I; next most common pattern = II; least common pattern = III.

Note that all chemicals could evoke activities with dominance in frequency and amplitude at 0.2–0.7 Hz, 1–2 Hz or 2–6 Hz. Activities evoked by onion and apple also elicit peakings falling into these ranges except the 2–6 Hz. This shows that this sensorimotor neuronal assembly is capable of generating patterns represented by these different groups of peak power frequencies.

As we previously reported and discussed in detail [39], the plot of Peak power I clearly indicates that peak power frequency increases when aversiveness decreases among this set of odorants. Contrary to that, the plot of group II revealed: (a) the PG occasionally (10–30% of cases), and

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### Table 1

The pattern of power increase

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Total</th>
<th>0.1–&lt;1 Hz</th>
<th>1–2.5 Hz</th>
<th>2.6–8 Hz</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>D.A. (cm)</td>
<td>a, b</td>
<td>c, d</td>
<td>Peak Frequency (Hz)</td>
</tr>
<tr>
<td>Ethanol</td>
<td>1.0±0.5</td>
<td>11, 71</td>
<td>I</td>
<td>9, 26</td>
</tr>
<tr>
<td>2-Butanol</td>
<td>2.9±0.6</td>
<td>5, 29</td>
<td>I</td>
<td>5, 21</td>
</tr>
<tr>
<td>2-Pentanol</td>
<td>5.3±1.1</td>
<td>8, 47</td>
<td>I</td>
<td>7, 27</td>
</tr>
<tr>
<td>Formic acid</td>
<td>6.4±1.0</td>
<td>11, 45</td>
<td>I</td>
<td>10, 30</td>
</tr>
<tr>
<td>Ammonia</td>
<td>8.5±1.0</td>
<td>5, 20</td>
<td>I</td>
<td>5, 17</td>
</tr>
<tr>
<td>Onion</td>
<td>3.5±0.6</td>
<td>4, 16</td>
<td>I</td>
<td>4, 14</td>
</tr>
<tr>
<td>Apple</td>
<td>N.W.</td>
<td>4, 15</td>
<td>3, 3</td>
<td>II</td>
</tr>
</tbody>
</table>

D.A., degree of aversion: determined from a number of active snails and expressed as stimulus-tentacle distance in cm at withdrawal reaction (cf. Ref. [36]). N.W., no withdrawal of tentacles. a, total number of preparations tested; b, total number of experiments made with the preparations; c and d, number of preparations and number of trials, respectively, made with the preparations, in which particular responses were elicited. The pattern of power increase is classified into three groups: I, most common pattern; II, next most common pattern; III, least common pattern. Degrees of aversion and frequencies are given as means ±95% confidence limits [45].

1 In one experiment peak power at 22–28 Hz was observed.

2 In one experiment peak power at 9 Hz was observed.
odor-nonspecifically, responded to repellant odorants (2-butanol, 2-pentanol, formic acid, ammonia and onion) with highest increase of power at 1–2 Hz; (b) The ganglion could sometimes respond to the attractive odorants (apple and ethanol) with frequency dominance at <0.6 Hz.

The plot of group III suggests that in a few cases the chemical stimulants tested could provoke activity patterns dominated by the 2.3–6.4 Hz oscillation.

The frequency center of initial activation systematically shifts towards the lower frequency with increasing aversiveness whereas it moves to a higher frequency — up to 2.5 Hz — by attractiveness. The border frequency for classifying odor quality from aversive to attractive may be ~1 Hz. Interestingly, presentation of attractive odorants immediately suppresses the activity in the range <0.5 Hz in a majority of the cases and this evidence also seems to conform the above observation.

### 3.3. Odorant-evoked 2–8 Hz activity and ~3 Hz oscillation

One of the most conspicuous activities of the odorant-induced responses of PG was the ~3 Hz oscillations that sometimes appeared as a quasi-sinusoidal oscillation. To study the nature of this oscillation, we examined the time records and the corresponding power spectra. In Table 2 we present all the cases observed together with concentrations, latencies and durations. In six out of 12 preparations, we observed this phenomenon.

We reported previously [39] an example of long lasting 2–8 Hz activity induced by an attractive odorant, ethanol. We present here two examples with aversive odorants from the same preparation (No. 115): one example with 2-pentanol (1:8 dilution; Fig. 3a,b) and another with formic acid (1:2 dilution; Fig. 4a,b). Fig. 3a (cf. Ref. [39] for time records and power spectral sequence) shows a case where a strong, intermittent ~3 Hz oscillation (marked with an asterisk) was elicited by 2-pentanol (1:8). The time evolution of the evoked activities are presented as a series of FAPs (each curve is an average from 20 epochs = 204.8 s). This peaking then slowly shifted back to a broad elevation at 0.5–2 Hz (Curve at 55 min) which appeared to be a close equivalence of the spontaneous ~1.3 Hz oscillation observed initially before odor application.

Fig. 4a,b presents the other case. The activity increased 1 min after presentation of formic acid (1:2) (Fig. 4a, B). Two minutes later this became a strong oscillation at ~1.3 Hz which lasted only for 30 s (Fig. 4a, C; Fig. 4b, second from bottom). Note that 4 Hz activity suddenly emerged for 1 min immediately after stimulus offset (Fig. 4a, D; Fig. 4b, fourth from bottom). Then, in another 8 min stable 3 and 6 Hz oscillations appeared persisting for 409.6 s (Fig. 4a, E; Fig. 4b, third and fourth from top). The 3 Hz oscillation persisted for at least another 7 min (Fig. 4a, F; Fig. 4b, two curves from top).

Here again, activity pattern changed starting with stimulus offset. It is particularly interesting that a clear 4 Hz oscillation of 1 min duration was discharged on removal of stimulus. It ought to be pointed out that this preparation originally showed conspicuously strong, spontaneous burst discharge at ~1.3 Hz (cf. Fig. 1, Prep. I). In such a case, initially, odorant stimulation seemed to nonspecifically activate this dominant component. As evidenced in these examples by the shifting of the frequency dominance from 1.3 Hz to ~3 Hz and back to 1.3 Hz, these two frequencies seemed to be interrelated with each other.

One remarkable phenomenon was that these ~3 Hz oscillations could sometimes respond to repellent odorants (2-butanol, 2-pentanol, formic acid, ammonia and onion) with highest increase of power at 1–2 Hz; (b) The ganglion could sometimes respond to the attractive odorants (apple and ethanol) with frequency dominance at <0.6 Hz.

### Table 2

Nonspecifically evoked 2–8 Hz activities and ~3 Hz burst oscillations of the Helix pedal ganglion

<table>
<thead>
<tr>
<th>Odorant</th>
<th>Prep./Exp.</th>
<th>Concen.</th>
<th>Latency (min)</th>
<th>Duration (min)</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>109a</td>
<td>Undil.</td>
<td>Immed.</td>
<td>&gt;45</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>109c</td>
<td>Undil.</td>
<td>0.5</td>
<td>&gt;30</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>109f</td>
<td>Undil.</td>
<td>0.5</td>
<td>12</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>110b</td>
<td>Undil.</td>
<td>10</td>
<td>24</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>110c</td>
<td>Undil.</td>
<td>4</td>
<td>23</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>113b</td>
<td>Undil.</td>
<td>0.5</td>
<td>&gt;30</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>115g</td>
<td>1:8</td>
<td>6</td>
<td>28</td>
<td>++ +</td>
</tr>
<tr>
<td>2-Butanol</td>
<td>115e</td>
<td>1:8</td>
<td>9</td>
<td>&gt;30</td>
<td>++ +</td>
</tr>
<tr>
<td>2-Pentanol</td>
<td>112c</td>
<td>Undil.</td>
<td>0.5</td>
<td>&gt;30</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>115d</td>
<td>1:8</td>
<td>8</td>
<td>&gt;55</td>
<td>++ ++</td>
</tr>
<tr>
<td></td>
<td>115k</td>
<td>1:16</td>
<td>4</td>
<td>12</td>
<td>+</td>
</tr>
<tr>
<td>Formic acid</td>
<td>109g</td>
<td>1:8</td>
<td>Immed.</td>
<td>16</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td>113c</td>
<td>1:8</td>
<td>2</td>
<td>14</td>
<td>+</td>
</tr>
<tr>
<td></td>
<td>115j</td>
<td>1:2</td>
<td>1</td>
<td>&gt;30</td>
<td>++ +</td>
</tr>
<tr>
<td></td>
<td>118b</td>
<td>1:2</td>
<td>3</td>
<td>11</td>
<td>++ +</td>
</tr>
<tr>
<td>Ammonia</td>
<td>115f</td>
<td>0.1%</td>
<td>1</td>
<td>&gt;35</td>
<td>++ +</td>
</tr>
<tr>
<td>Onion</td>
<td>120d</td>
<td>3</td>
<td>13</td>
<td></td>
<td>++ +</td>
</tr>
</tbody>
</table>

Preparations are designated by numbers and trials by alphabets. The observations were based on the survey of the time records and power spectra. The expression of activity is relative. Note that except in two cases the bursting occurred after a certain latent period (from 0.5 to 10 min) and lasted long (from 11 to 45 min) often emerging intermittently.
odor-nonspecific evoked activities were long-lasting either intermittently (Fig. 3a, b) [39] or continuously discharged (Fig. 4a, top two panels). Note that in both cases this oscillation emerged after removal of stimulus, more or less, starting with the stimulus offset.

3.4. Latent period

Concerning the term ‘latency’ we need to draw special attention to a phenomenon that we consistently observed during the very first period after odor presentation. That was a short period (0.5 to a few minutes) of sudden quiescence and we observed it not only in PG, but in PC and VG as well [36, 38, 39]. It occurred immediately (<3 s) after odor application and before any drastic alteration of activity pattern appeared; a typical example is shown in Fig. 3c. As demonstrated by the time records (middle), the slow waves and spikes of the ongoing activity of PG became drastically suppressed on stimulus application, but in a few minutes spiky, multicomponent activity was discharged (top). As summarized in Table 2, the ~3 Hz oscillation was evoked after a short latent period (0.5 to a few minutes), in the initial phase of which the ongoing activity became quiescent.

3.5. RMS amplitude

Odorant-evoked ~2 to ~3 Hz oscillations were often discharged for a long time intermittently [36, 39]. To examine how individual frequency components fluctuate with time, we processed the signals of some representative cases (cf. Table 2) with different pass-band filters. We then calculated RMS voltages (µV) of the filtered signals for all 20 epochs and the percent changes of the average RMS voltage from those of prestimulus activities (controls). We demonstrate in Fig. 3b, as an example, the experiment (Fig. 3a) where stimulation with 2-pentanol (1:8 dilution) evoked long-lasting intermittent burst oscillations in the

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Fig. 3. Odorant-evoked ~3 Hz oscillation of the *Helix* pedal ganglion. Stimulant: 2-pentanol (1:8 dilution). The same experiment as 115d in Table 2 and the same preparation as preparation I in Fig. 1. The time records and single power spectra [39]. (a) Each FAP was computed from a 204.8 s sample. Note that a dominant peak around ~3 Hz (marked with *) emerged after, and starting with, the stimulus offset and remained stable for long. Note also that the frequency center later shifted towards ~1 Hz. This preparation incidentally had shown strong ~1 Hz spontaneous oscillation (Fig. 1-I). (b) Time evolution of the ~3 Hz and other components. Percent changes of the RMS-voltages of different frequency components from those of prestimulus activity (control). Note that burst activities, alternating with quiescent periods, recurred mainly at a rhythm of every 9–13 min. Strong increase and robustness of the 0.5–8 Hz activity, particularly the 2–4 Hz component, were evident in the histograms. (c) Initial quiescence caused by odor application. This sequence was observed three out of four times with odorants and not in four trials without odor. Note that slow waves and spikes of ongoing activity (bottom) became suddenly suppressed by application of the stimulus (middle). In this case, the quiescence lasted 1.5 min. Increased activity became then more visible, as the records from one of the early evoked patterns (top) show.
2–8 Hz range. As shown by the histograms, the frequency component of 0.5–8 Hz was particularly robust during the later period.

Note that the oscillatory burst seemed to occur at a certain rhythmical cycle, even after removal of the stimulus: Fig. 3b shows a cycle of 9 to 13 min. Interestingly, we observed this type of intermittent discharge in the Helix CNS mainly with alcohols, such as ethanol, 2-butanol and 2-pentanol.

4. Discussion

In the present study we address the following questions on the nonspecific, but unique power peaks evoked by odorants in the Helix PG. (1) What are their features? (2) Are they comparable to the activities found in the motor systems of the other invertebrates? (3) To what functions can they be related? (4) Are the nonspecific responses evoked by odorants in the snail CNS comparable to those detected in the brains of other species by different sensory and/or cognitive inputs?

Our experimental approach differs from most others reported so far, particularly in the following aspects: (i) use of gross steel electrodes for recording population activities; (ii) lengthy stimulation (10 min); (iii) long observation during and after stimulation; (iv) power spectral presentation of dynamical evolution of odorant evoked activity patterns; (v) estimation of peak power frequency of response pattern by FAP (obtained from signals averaged in the frequency domain). As suggested by our results [36,38,39], it is important to give either repeated or continuous odor samplings and to record dynamically evolving activity patterns for a prolonged time. The recent report by Stopfer and Laurent [42] strongly supports this idea.

As shown in the previous studies [36,38,39], in each of the three centers, PC, VG and PG, the odorant-induced activity pattern is largely odorant-specific and related to the nature of the odor and behavioral output. In PG, however, the odor input elicits a larger variation from trial to trial and preparation to preparation in response activities compared with PC and VG.

To answer the first question, we classified the odorant-evoked activity patterns of PG as commonest, least usual and intermediate in incidence and specially focused on certain lesser, but important and unique, activities of this ganglion generated by odor input. Three main frequency components represented by power peaks at <1 Hz, 1–2 Hz and 2–8 Hz seem to feature the response activities. (a) The aversive odorants induce odorant-specific patterns represented by peak power frequencies at <1 Hz. (b) The oscillation at ~1 Hz, which exists intrinsically in the Helix PG, can be specifically enhanced by appetitive odors. It is also nonspecifically evoked by aversive odorants, but much less frequently. (c) The 2–8 Hz component always
accompanies the odorant-evoked activities of the PG either as the second or third strongest component, or in the form of conspicuous, long-lasting ~3 Hz oscillations. We may therefore consider it as one of the elementary oscillations that are involved in the processing of signals in this ganglion. (d) The nonspecific odor evoked 1–2 Hz and ~3 Hz activities, and the intrinsic ~1 Hz activity of the PG seem to be interrelated by a degree of mutual exclusion.

We applied the additive periodogram [6] to some of the data presented in Fig. 3a as FAPs for the purpose of comparison. It shows that power spectrum and FAP peaks in the snail, between one and 2.5 Hz do not in fact
Fig. 4. Odorant-evoked ∼1.3 Hz and ∼3 Hz oscillations of the Helix pedal ganglion. An example of evoked ‘continuous’ ∼3 Hz oscillations. Stimulant: formic acid (1:2 dilution). The same preparation as Preparation I of Fig. 1 and the same trial as 115j in Table 2. (a) Power spectral series, left, and time records, right. Calibration: 110 μV for C and 50 μV for the rest. The positions in the power spectra corresponding to the time records are indicated by arrows. First oscillatory response activity appeared 1 min after stimulus onset, then evolved to a strong ∼1.3 Hz burst of 1 min duration and continued as dynamic multicomponent fluctuations. Note that 4 Hz oscillation of 1 min duration was elicited starting with stimulus offset (third panel from bottom, left). Note also that the curve at stimulus offset depicts a sharp peak at 4 Hz reflecting the 4 Hz oscillation of the power spectra, although this 4 Hz oscillation could hardly be recognized by eye in the time records due to other superimposing components. The cellular population then started to discharge spike oscillation at 3 and 6 Hz (4th and 5th panels, left, and 5th and 6th panels, right). (b) The corresponding Frequency–Amplitude Plots (FAPs). Note that these FAPs clearly demonstrate dynamics of the frequency components: **4 Hz and *3 Hz. Note also that the 4 Hz activity (a-D) and the 3 Hz oscillation are reflected as small peaks (marked with ** and *, respectively). The strong burst evoked by formic acid (1:2 dilution) is here shown as the three dominant peaks at 0.7, 1.3 and 2 Hz.
transients. The power spectrum is not a reliable indicator of rhythms. The 3.3 Hz activity is clearly a rhythm, because it satisfies such a definition, by inspection, being large enough to see above the wideband background, and appears of course as a peak in the periodogram as well as the power spectrum (unpublished data).

In the following we discuss some important properties in terms of ‘population responses’.

4.1. Dynamics of population responses

Here, we point out two important features of odorant-induced responses of the Helix PC, VG and PG. First, in the early stage olfactory activities are either little changed or, more often, become rather quiescent before they evolve dynamically with time. For ca. 0.5 to a few minutes after stimulus onset, depending on the intensity, the activity first becomes quiescent with amplitude of low-frequency fluctuations decreased and spikes suppressed [36,39]. Second, the oscillatory frequency of the response seems to be related to the reaction time of the superior tentacles suggesting the odorant dependency, but the latency of the odorant-specific oscillatory response is usually much longer (>30 s) than the reaction time (for instance, ≤1 s for formic acid and 6 s for ethanol) [36]

There are two inevitable questions to be answered about these phenomena. (1) What significance has the initial quiescence after stimulus onset? (2) Why is the latency of the oscillatory response much longer than the time of the tentacle withdrawal reflex?

We presume that this period of quiescence may be a necessary state for synchronizing population activities to bring the neurons to oscillate more coherently for precise categorization in the entire central pattern-generating circuit [41]. A recent study on population dynamics of locust antennal lobe neuron assemblies [42] shows that repeated or prolonged odor sampling increases spike and interneuronal oscillatory coherence and improves performance in categorization. Their finding not only supports our idea, but our method of lengthy stimulation and prolonged recording as well. Interestingly, in the mammalian motor cortex, short (1 s) inhalation of odor causes low-voltage, fast (‘desynchronized’) activity — a state of EEG arousal [16]. Whether we can relate the initial quiescence of PG to this arousal or not needs additional debate. Regarding the discrepancy between the short tentacle reaction time and the delay in odorant-dependent oscillation, Teyke and Gelperin [43] report similarly that a clear change in LFP oscillation in the Limax PC lobe occurs, if ever, long after the external peritentacular nerve (ePTN) discharge that is normally evoked by a conditioned odor with a latency of ~2.5 s after the onset of stimulus.

4.2. ~1 Hz oscillations, spontaneous and induced, in central pattern generating circuits

Regarding the second question, we compare the ~1 Hz oscillations (by a broad definition, such as a similar form repeating more than three or four times with a period that fluctuates less than 30% on either side of the mean). Presumably these peaks are from irregular or single
activities of the *Helix* PG with those of other invertebrates below.

We may compare the ~1 Hz spontaneous discharge with the similar oscillations observed in the lobster pyloric neuron network [17] and the *Limax* PC-BG circuit [13]. The rhythm of the pyloric network that generates a rhythmic motor pattern at ca. 1 Hz can be modulated by monoamines. A hard-wired oscillatory network built according to this biological model generates similar oscillations [27]. In the *Limax* network [13], a buccal cell (BC) fires at 1.8±1.4 Hz and electrical stimulation of this BC increases frequency of LFP oscillation of PC from 0.7 to 1.4±0.4 Hz. In addition, stimulation of PC with NO increases the frequency of the LFP oscillation and also activates BC spike activity at 1–2 Hz. This means that the neurons that are functionally involved in motor activity (the pylorus or the pharynx movement) fire spontaneously at a frequency of 1–2 Hz, similar to that observed in the *Helix* PG by gross recording.

Gelperin et al. [13] also demonstrate that the *Limax* pedal cells spontaneously fire spikes at ~1 Hz, but they are only weakly coupled to LFP oscillation of PC. A similar finding has also been reported on the *Aplysia* feeding circuit [31], that is, low-frequency firing (0–5 Hz) of cerebrobuccal interneurons, that occurs during a buccal motor program, has an output in the CG and this output is electrically coupled to the cerebro-buccal-interneurons and to a cerebro-pedal-neuron.

Furthermore, the interneurons of the locust mesothoracic ganglion show an oscillatory activity pattern at 2–3 Hz that is coupled to the movement of the middle leg [44].

It can be inferred from these observations that the 1–3 Hz spontaneous discharge is strongly connected with motor activity that involves the feedback mechanism of the procerebro-cerebro-buccal or -procerebro-cerebro-pedal circuit.

Command cells (several pairs of neurons in the cerebral ganglion) make direct and indirect outputs to neurons in the PG, including motor neurons, ‘a central pattern generator circuit’, and modulatory neurons that enhance muscle contractions during locomotion [12].

Concerning the third question we address the possible functions of the unique oscillations in terms of locomotion function and short-term memory function.

4.3. Locomotion function

It is notable that we can, in the *Helix* PG, record spontaneous FP activities that seem equivalent to the other invertebrate models. The present observations suggest that the ~1 and ~3 Hz oscillations of PG may also be processed in the cerebro-procerebro-pedal feedback circuit.

A question was raised: what type of oscillatory correlations are there between PC and PG? We carried out some pilot experiments to simultaneously record activities from the PC and PG applying different odorants. This time PC potentials had to be recorded on the ventral side, not on the dorsal side as done previously, for technical reasons. Fig. 5 illustrates an example with an onion stimulus. This odorant evoked both in PC and PG activity patterns with peak power frequencies at ~0.4 Hz (cf. Table 1 and Fig. 1). The patterns were similar, but the activity <2 Hz was much stronger in PC than in PG. This is interesting since it suggests that, as we predicted earlier, odor information is processed first in PC as a specific activity pattern, as shown here, represented by a frequency of ~0.4 Hz, and propagates to PG where it activates the population network, but probably weakly coupled. Another notable point was that, after stimulus offset, only in PG the 2.5–10 Hz activity was elicited whereas in PC it was no longer generated. This observation again suggests that the 2.5–10 Hz activities are characteristic for this locomotion center.

It is known that forward movement of the snail is made by so-called locomotion waves (pedal waves) of the pedal muscle. The excitatory impulse from the PG is transmitted over the pedal nerves and the sole plexus, activates the muscle fiber for contraction, and produces pedal waves [7,24,29]. The pedal waves travel constantly from the back to the front, eliciting a contraction rhythm of 0.35–0.6 Hz in the *Helix* sole muscle during locomotion [24]. Notably, in our model, the PG spontaneously generates FP activity with frequency dominance at 0.3 and 0.6 to ~1 Hz. This strongly suggests that these frequencies are related to maintaining the peristalsis of the foot. It remains to record the activities simultaneously from the pedal muscle and the PG to verify whether the above prediction is justified.

4.4. Short-term memory function

Once odor is categorized (or encoded) and localized, short-term stimulus-specific sensory memory is constituted and decision is made for ‘approach’ or ‘avoid’ via the feedback circuit involving the CG.

The present study shows that the ~3 Hz oscillations or the 2–6 Hz activities evoked in the *Helix* PG are not odorant-specific. Concerning these activities, we particularly point out that they occur frequently starting with stimulus offset and last long thereafter (>30 min). We presume that this is a subclass of memories; the short-term sensory memory (or sensory template or ‘encoded information’) triggers the scored template and excites whole motor systems controlling posture or locomotion. As known for locust flight, reafference primarily plays a role in controlling the average excitation of the motor pattern generator (central programming by comparison of sensory feedback with a centrally stored goal pattern) [8]. In our previous report [36] we proposed a relationship to memory function of similar, intermittently enduring oscillation (~2 Hz) that are evoked in the *Helix* VG by delivery of ethanol.

4.5. Function of the low-frequency oscillations in the brain

Here we discuss the results from a comparative stand-
Fig. 5. Onion-evoked activities of the *Helix* procerebrum (PC) and the pedal ganglion (PG). Simultaneous recordings. Channel 1: PC; Channel 2: PG. The power spectral sequence on the left and the time records on the right. Each panel of the power spectra represents data of 40 epochs (~409.6 s), starting at the bottom. The activities simultaneously increased in both PC and PG by odor input, as the time records showed. Note that onion evoked activity pattern, specific to this odor, with peak power frequency at ~0.4 Hz. This was estimated from the corresponding FAPs (the curves not shown). Compare also with Table 1 and Fig. 1. As long as stimulus was present (7 min), the patterns of evoked activities in both PC and PG were similar, but the activity of PC below 2 Hz was much stronger than that of PG, as the power spectra indicated. Note that the burst of PG at 3.5 min after stimulus onset extended up to 10 Hz while that of PC barely up to 8 Hz. Note also that in PG the 2.5–10 Hz activity was elicited even after removal of stimulus, whereas in PC no such activity was generated.
point to answer the fourth question we posed. The present findings lend support to the proposition that the frequency range $<8$ Hz is most commonly involved in processing of odorant information in the *Helix* central pattern generating circuit for movement of foot and body wall. Low-frequency components of the range $<8$ Hz are evoked by sensory stimulation in both mammals and fish [5,9] and by electrical stimulation in the invertebrate [35,37], and in...
turtle [32]. The earlier findings from experiments carried out in our laboratory with humans (odd-ball, missing tones, hearing threshold, miss-match-negativity) show that activities in the <4 Hz range are induced by various cognitive inputs and considered to be involved in expectancy, decision making, signal detection and detection of change in physical features of input [3,4,30]. It is noteworthy that low-frequency field potential fluctuations similarly take part in the olfactory information processing of the Helix central circuit. Whether or not the relevant processing is ‘cognition’ or ‘recognition’ or the subsequent emergence of behaviorally relevant activity pattern in the brain of the invertebrate can be debated. We do not have evidence to choose among alternatives.

Our model seems to be appropriate for studying dynamics of the whole olfactory network system from a comparative standpoint. The snail olfactory system can be considered to represent olfactory systems in general [10].

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