

Spectro-temporal characteristics of single units in the auditory midbrain of the lightly anaesthetised grass frog (*Rana temporaria* L.) investigated with tonal stimuli

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Responses were obtained from 112 auditory neurons in the midbrain of the grass frog in response to sequences of tones. Their spectro-temporal sensitivities (STS) were determined by a second-order cross-correlation technique. For the majority of units the shape of their action potentials, the degree of timelock to the stimulus and the recording sites were obtained. Two stages of information processing could be distinguished. One was characterized by short latencies (<30 ms), strong timelock to the stimulus and many of these units had axon-like action potential waveforms. They were localised in the ventral part of the principal nucleus from the torus semicircularis and in the transition region between laminar and principal nucleus. The other stage comprised units, found all over the torus, with longer latencies, and a weaker timelock to the stimulus. Several units which were predominantly found in the central part of the torus, especially the magnocellular nucleus, showed a broad or multiple STS.

Within the principal nucleus a weak tonotopy was found, the dorsoposterior part being sensitive to lower frequencies, the ventroanterior part to the higher frequencies. Binaural-interaction properties are discussed with respect to the eardrum coupling through the mouth cavity. An organisational plan for the torus semicircularis is proposed.

Key words: spectro-temporal properties; second-order cross-correlation; torus semicircularis; frog; tonal stimuli.

Introduction

Only about 30% of the auditory midbrain neurons in the lightly anaesthetised grass frog respond in a sustained way to minutes' long stationary gaussian wide-band noise. The spectro-temporal characteristics of these neurons were investigated by second-order cross-correlation between the noise stimulus and the action potentials [17]. In order to investigate the spectro-temporal properties of a larger proportion of

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auditory midbrain neurons, a modified second-order cross-correlation procedure [4] was applied to responses to tonal stimuli. Both second-order cross-correlation procedures provide a combined spectral and temporal description of the response properties of the neuron. In previous papers [11,17] it was argued that for many units such a combined description must be preferred above a separate description of the tuning and the temporal, generally called dynamic, properties of the neuron.

Recently, Walkowiak [25] reported properties of neurons in the torus semicircularis of immobilised grass frogs in response to 500-ms noise bursts, tone bursts, and species-specific vocalisations. Tuning curves and two-tone interaction were studied, which confirmed for the grass frog the existence of three ranges of frequency sensitivity in which the auditory system of higher anurans can be divided [7]. Furthermore, the effect of stimulus intensity and the selectivity for species-specific vocalisations were studied.

In the present study besides the spectro-temporal sensitivities three other neuronal characteristics were determined: the timelock of the action potentials to the stimulus, the binaural interaction properties and the type of the action potential waveform [10]. The spectro-temporal sensitivity and these three characteristics will be related to each other and to the recording site in the torus semicircularis. It appears that the tonotopy found in an evoked potential study [20] is probably only due to a weak tonotopical organisation of the principal nucleus. Complex tuning properties are found in the central parts of the torus semicircularis, particularly in the magnocellular nucleus. Furthermore, it appears possible to distinguish two stages of information processing in the torus semicircularis. The first one consists of units with latencies shorter than 30 ms and a strong timelock to the stimulus, whereas the second consists of units with latencies longer than 30 ms and a weaker timelock of the stimulus. Based on these results a global organisation of the torus will be proposed.

Methods

Adult grass frogs from Ireland were lightly anaesthetised (MS 222) and recordings in the torus semicircularis were made using stainless-steel microelectrodes which penetrated the intact dura. The animals were kept at a temperature below 18°C, generally at about 15°C, in a sound-attenuated room. All details about the preparation, the recording of single-unit activity, histological procedures and data acquisition have been described in a previous paper [17]. The tonal stimuli were generated under computer control [4,17]; their exact characteristics will be described. A closed-sound system was used. During recordings the oral cavity was shut.

The tonal stimulus ensembles

The stimulus ensembles in their most general form consisted of rather long (cf. Table I) sequences of tone pips. One tone pip can be described as:

$$s(t) = a(t) \sin 2\pi ft$$

in which

$$a(t) = A \left(\frac{t}{\beta} \right)^{\gamma-1} \exp\left(-\frac{t}{\beta} \right) \quad t \geq 0$$

$$= 0 \quad t < 0$$

A is a constant determining the maximum peak equivalent, sound pressure level of the tone pip; f is the frequency of the tone. The envelope $a(t)$, is determined by the duration constant β and form constant γ .

A basic property of the stimulus ensembles is that the frequency values f were pseudorandomly selected from 127 or 255 logarithmically equidistant values between either 125 and 2000 Hz or 250 and 4000 Hz depending on the best frequency of the neuron. The maximum amplitude A of the tone pips was kept constant or otherwise randomly selected from 31 or 127 linearly equidistant values. When 31 amplitude values were selected the peak intensity of the tones varied between 59 and 89 dB SPL; when 127 values were selected, between 47 and 89 dB SPL. Thus in case 255 frequency values and 127 amplitude values were selected, each amplitude–frequency combination occurred once in the stimulus ensemble but a given frequency was presented 127 times.

Although the various frequency and amplitude values were presented in pseudorandom order, this did not guarantee that the frequency of one tone was strictly independent from that of previous or following tones [4]. This residual correlation in the pseudorandom sequences, however, appeared not to influence the measured latencies or best frequencies as could be concluded from comparisons of responses to stimulus ensembles with different statistics. Nevertheless, phenomena like post-activation suppression were not investigated, since these require strict independence between the frequencies of successive tones.

1: The first stimulus ensemble consisted of short tone pips with $\beta = 1.45$ and $\gamma = 3$. These tone pips were truncated after 16 ms when the envelope had decreased to 0.36% (–49 dB) of its maximum. At that moment the next tone pip started. So, the onset intervals between the tones were 16 ms. Frequencies were selected from 255 values and amplitudes from 127 values (see Fig. 1). A detailed description of this SE was presented earlier [3]. It lasted 8 min 38 s.

2: Occasionally a much shorter stimulus ensemble was used based on 127 frequency values and 31 amplitude values. When onset intervals of 16 ms were used, this stimulus ensemble lasted 1 min 3 s. When onset intervals of 32 ms were used, it lasted 2 min 6 s.

These stimulus ensembles will be called *short-interval stimulus ensembles*. The cross-correlation procedures in this study require that the probability of occurrence of an action potential is the same every time the same tonal stimulus is presented. A response fulfilling this requirement will be called a *sustained response*. It appeared that 52% of the neurons in the torus semicircularis did not respond in a sustained way to these short-interval stimulus ensembles (SEs). They either did not respond at all to these short-interval SEs or fired only at the start of the SE after which the firing rate gradually decreased or became zero. In order to overcome this habituation-like behaviour, stimulus ensembles with longer intervals between the onset of

Gamma - tones

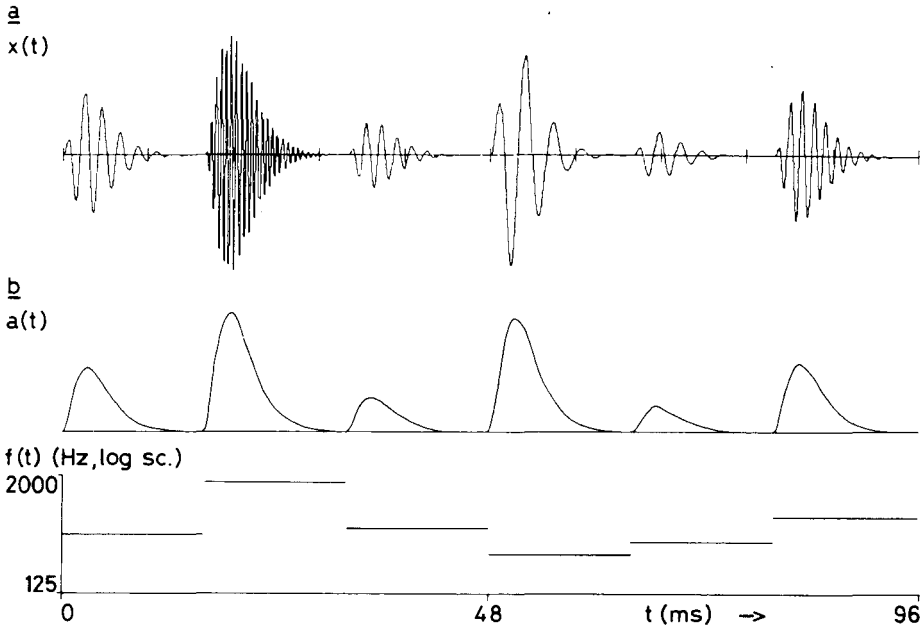


Fig. 1. Part of the tonal stimulus ensemble (from Aertsen and Johannesma [3]).

the tone pips were constructed. The tone pips of these *long-interval stimulus ensembles* all had fixed peak amplitudes of 89 dB SPL.

3: One such stimulus ensemble with $\beta = 1.45$ ms and $\gamma = 3$, as in the former stimulus ensembles, was constructed from 255 frequency values with onset intervals of 128 ms. One sequence of 255 tones was repeated 9 times, resulting in a duration of 4 min 54 s for this stimulus ensemble.

4: The last stimulus ensemble consisted of tone pips with $\beta = 4.35$ ms and $\gamma = 3$. So these tone pips lasted three times as long as the tone pips of the former stimulus ensembles. The onset intervals now were 1000 ms and 127 frequency values were selected. This sequence of tones was repeated 4 times, so that the stimulus ensemble lasted 8 min 28 s. It appeared that 83% of the auditory units responded in a sustained way to this stimulus ensemble, which was verified by comparing the number of action potentials produced during subsequent presentations of identical sequences of tone pips. A survey of the SEs is presented in Table I.

Spectro-temporal characterisation of neurons

Second-order cross-correlation procedures are based on the concept of stimulus ensemble (SE) and pre-event stimulus ensemble (PESE) [2,16-18]. The SE is the complete set of stimuli presented to the neuron. The PESE is the subset of the SE consisting of those elements that precede an action potential. In cross-correlation

TABLE I
CHARACTERISATION OF STIMULUS ENSEMBLES (SE)

SE	N_f	N_A	β (ms)	Onset interval between tones (ms)	Duration of SE	% Sustained responses
I	255	127	1.45	16	8'39"	48
II	127	31	1.45	16 or 32	1'3" or 2'6"	48
III	255	1	1.45	128	4'54"	66
IV	127	1	4.35	1000	8'28"	83

N_f = number of different frequency values; N_A = number of different amplitude values; SE = stimulus ensemble; β = duration parameter of tones.

procedures average functionals of the PESE are compared with the average functionals of the SE [2]. The cross-correlation procedure used in this study was developed by Aertsen et al. for tonal SEs [4]. A second-order functional with frequency and time as arguments is defined for narrow-band (tonal) stimulus ensembles. It closely resembles the dynamic spectrogram, or sonogram. This functional is averaged over the PESE. For simplicity the results of these procedures can be considered to equal the average dynamic spectrogram of the PESE and will be called the *spectro-temporal sensitivity* (STS) under tonal stimulation of the neuron.

The result of one such averaging procedure is shown in Fig. 2. The STS of this unit is characterised by a clear dark region, which represents a high intensity. This dark region at about 14 ms before the spike extends in the frequency range of 840–1540 Hz. Its maximum value was obtained at 1140 Hz and will be called the *best frequency* (BF), because it implies that the unit fired best to tones of this frequency. The dark region starts at BF at 19 ms before the spike, indicated by an

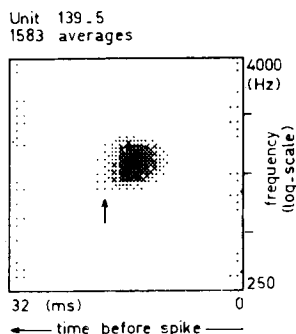


Fig. 2. The spectro-temporal sensitivity of a unit with best frequency of 1140 Hz and latency of 19 ms. The arrow indicates the latency. The dots at about 0 and 32 ms indicate the presence of tones following and preceding the tones that elicited the spikes. The SE consisted of short tones with onset intervals of 16 ms (SE I).

arrow. This is the *latency* of this unit to these tones. The dots at about 0 and 32 ms, i.e. about 16 ms before and after the dark region, represent the intensity distribution of the tones that followed and preceded the tones that elicited the spikes.

The existence of a stimulus–event relation

The existence of a stimulus–event relation was investigated by determining the cross-coincidence histogram (CCH) of two spike trains obtained upon two successive presentations of identical SEs [1,17]. Furthermore, the width of the CCH at half its maximum was used as a measure for the timelock of the spikes to the stimulus.

Action potential waveform

Action potential waveforms in the torus semicircularis of the grass frog can be divided into four different types [10,11]. The first type, *type I*, consists of short (about 2.5 ms) action potentials with a small positive first phase, a fast rising negative second phase, and a small positive third phase. The second type, *type II*, consists of longer lasting (about 5 ms) biphasic action potentials, that have a clear negative first phase. Units with waveforms of type I and II have relatively low amplitudes, generally lower than 200 μV . Waveforms of type III and IV generally have amplitudes higher than 200 μV . *Type III* has two or three phases with a clear positive first phase. It lasts from 5 to about 7 ms. The last type, *type IV*, are very long lasting (over 7 ms) action potentials with three or four phases, the first one being positive.

Binaural interaction

In most cases results will be presented that were obtained under binaural stimulation. The results for contralateral stimulation will be presented only for those units in which contralateral stimulation produced many more spikes than binaural stimulation. In addition, by comparing responses to binaural, ipsilateral and contralateral stimulation units were classified as OE-units, EO-units, EE-units and EI-units using the nomenclature of Feng and Capranica [13,14].

Results

Units with sustained responses to tonal SEs

Responses to tonal stimuli were recorded from 112 auditory units in the mid-brains of 46 grass frogs, *Rana temporaria* L. For 90 of these units responses were obtained for one of the stimulus ensembles with short intertone intervals. 43 (48%) of these units responded in a sustained way to these short-interval SEs, in 42 of which the existence of a stimulus–event relation was established by determining the cross-coincidence histogram between the spike trains obtained during two successive presentations of the same stimulus ensemble. So 47 units did not respond in a sustained way to these stimulus ensembles.

The stimulus ensemble with intertone intervals of 128 ms was presented to 43 units and evoked a sustained response in 26 of these units, in 24 of which the

existence of a stimulus–event relation could be established. The stimulus ensemble with intertone intervals of 1000 ms was presented to 60 units, 45 of which responded to this ensemble in a sustained way. The two latter types of SE were in many cases only presented when no sustained response to the short-interval SES could be obtained. Because all units that responded in a sustained way to the short-interval ensembles invariably did so to the long-interval ensembles, it is concluded that 66% and 83% of the auditory units in the midbrain of the frog respond in a sustained way to tonal stimuli presented with intervals of 128 and 1000 ms, respectively (cf. Table I).

The distribution of BFs

For the units in which a sustained response to one of the SEs could be obtained, second-order cross-correlation between the tonal stimuli and the neural events resulted for 83 units in the determination of their spectro-temporal sensitivities. To 69 of these units one BF could be attributed, while in 14 the spectro-temporal sensitivity consisted of more or less separated regions. Units with one BF will be called singly tuned; the other ones broadly tuned. The distribution of BFs of units with one BF is shown in Fig. 3. A separation can be observed around 900 Hz, which probably reflects the division between units receiving input from the amphibian papilla (BF < 900 Hz) and units receiving input from the basilar papilla (BF > 900 Hz) [7,25]. Units with BFs < 400 Hz will be called low-frequency units, with BFs between 400 and 900 Hz mid-frequency units, and with BF > 900 Hz high-frequency units. The division at 400 Hz is based on the results of Walkowiak [25], who established that most units in the torus of the grass frog with BF < 400 Hz showed two-tone suppression, which is the crucial test for a unit to be of low frequency [7].

The distribution of latencies

It appeared that the response latencies obtained for short-interval SEs were in general somewhat longer than those obtained for SE with longer intervals. This was especially evident in units with relatively long latencies. The relation between the latencies obtained under these conditions is given in Fig. 4. Because the long-interval SEs were often used only when a sustained response to short-interval SEs could not be obtained, for many units latencies were only available for short interval SEs. In

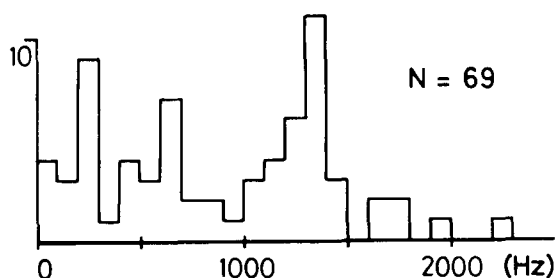


Fig. 3. Distribution of the best frequency of 69 singly tuned neurons.

this case the latencies for long-interval SEs were estimated from the regression line of Fig. 4 in order to obtain a latency distribution for all units under the same stimulus condition. This distribution is given in Fig. 5, and shows a dip at around 30 ms. This warrants the subdivision into short- and long-latency units. It appeared that short-latency units were overrepresented in the population of high-frequency units (χ^2 -test, $P < 0.05$). 11 of the 14 broadly tuned neurons had long latencies.

Latency and width of the cross-coincidence histogram

Fig. 6 shows the cross-coincidence histograms (CCH) of a short-latency and a long-latency unit. For the short-latency unit a relatively sharp peak can be observed, whereas the long-latency unit shows a much broader peak. The width of the CCH at half its maximum amplitude is taken as a measure for the lock of the neural events to the stimulus. This halfwidth is plotted against latency in Fig. 7. It is clearly shown that all units except two with latencies shorter than 30 ms had CCHs with a halfwidth less than 6 ms. Units with latencies longer than 30 ms had CCHs wider than 5 ms.

The spectro-temporal sensitivities

The spectro-temporal sensitivities of three *short-latency units* are shown in Fig. 8. A low-frequency unit, BF = 310 Hz, is shown on the left (Fig. 8a), a mid-frequency unit, BF = 680 Hz, is shown in the middle (Fig. 8b), and a high-frequency unit, BF = 1610 Hz, is shown on the right (Fig. 8c). The latencies at BF were 16 ms, 24 ms and 16 ms, respectively. It is observed that latencies are slightly frequency dependent.

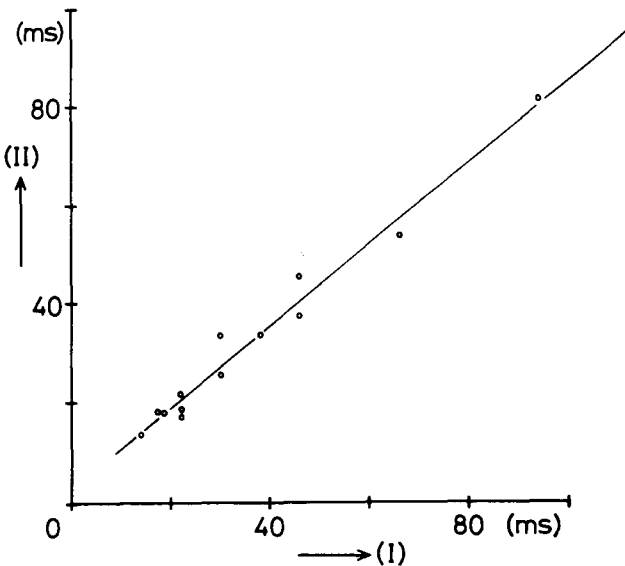


Fig. 4. Relation between the latency to tones with 1 s onset interval (I) and the latency to tones with 16 or 32 ms onset intervals (II). The regression line fulfils the equation: $lat_{II} = 0.834 lat_I + 2.56$.

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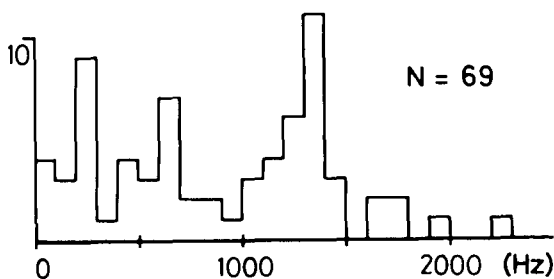


Fig. 3. Distribution of the best frequency of 69 singly tuned neurons.

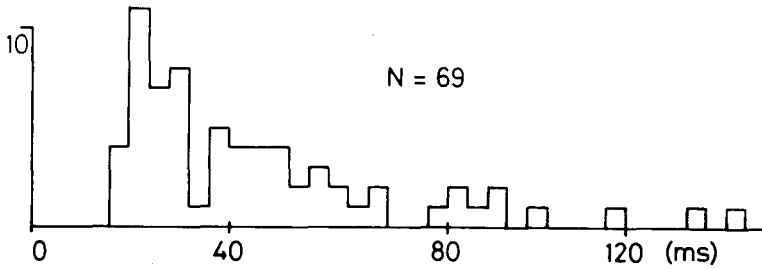
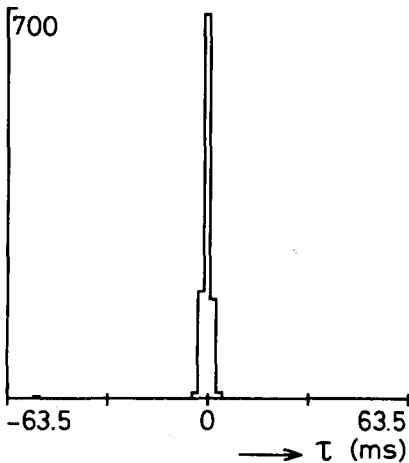


Fig. 5. Distribution of the latencies of 69 singly tuned neurons.

dent, becoming longer for frequencies further away from BF. STSs of this type are rather general among short-latency units. Sometimes a very strong saturation could be observed (Fig. 9). This consisted of an STS in which the maximum intensity was constant over a considerable frequency range. In this range the latency was constant. Saturation was almost exclusively found in short-latency units. Because most of these units responded in a sustained way to stationary wide-band noise, the STS could be compared with the spectro-temporal properties under noise stimulation. Here the stimulus intensity per Hz was much less and no saturation was observed

a:
Unit 161 - 4
1123 coincidences



b:
Unit 167 - 4
784 coincidences

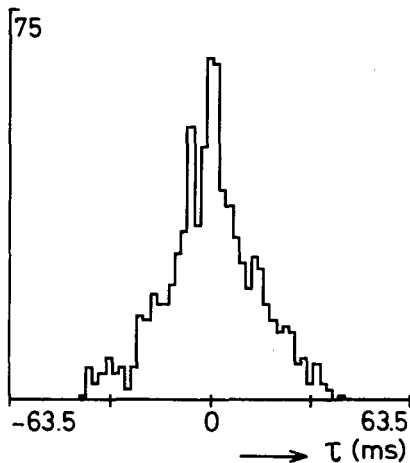


Fig. 6. Cross-coincidence histograms (CCH) of a short-latency unit (a) and a long-latency unit (b) obtained under stimulation with a tonal SE with onset intervals of 128 ms. The binwidth of the histogram is 1 ms. So the halfwidth of the first CCH is about 1 ms whereas the halfwidth of the second CCH is 9 ms. The firing rate of the unit shown in a was 4.02 spikes/s, the unit shown in b had one of 3.84. The total period in which the analysis was done lasted 260 s.

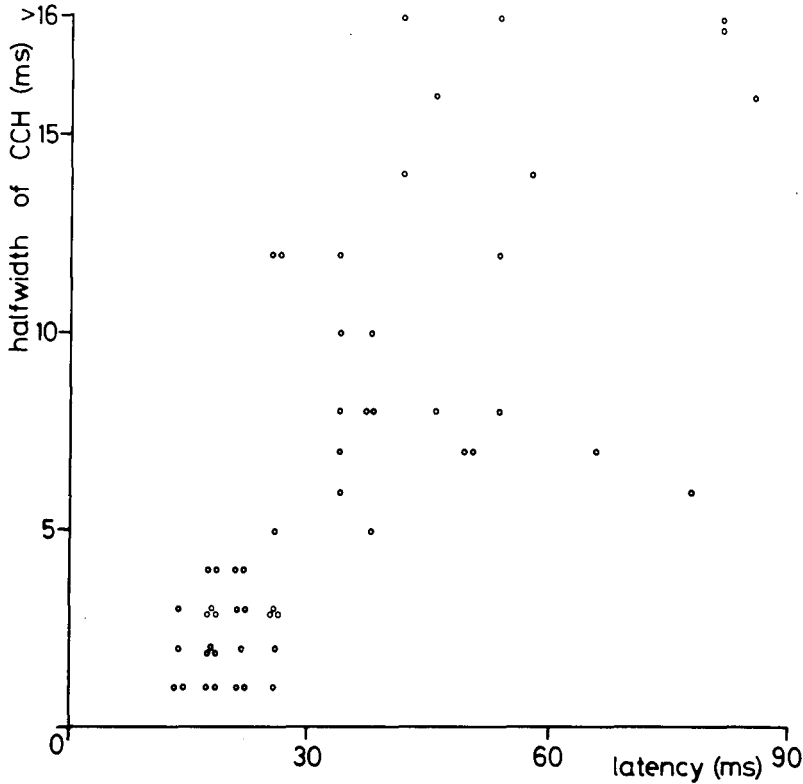


Fig. 7. Relation between the latency of a unit and the halfwidth of its cross-coincidence histogram.

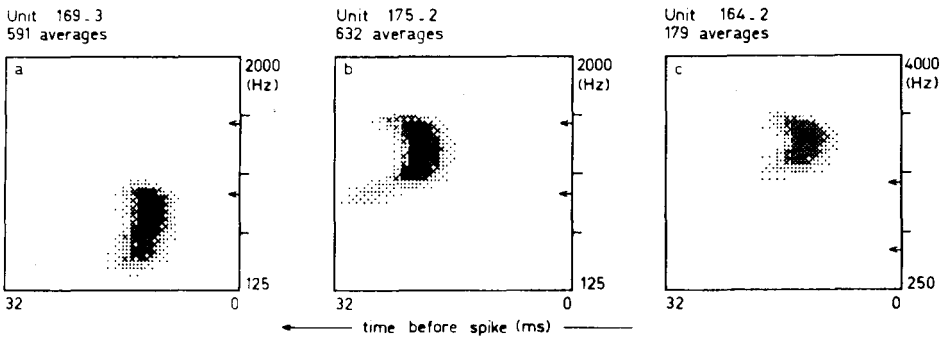


Fig. 8. Spectro-temporal sensitivities of three short-latency units: one low-frequency (a), one mid-frequency (b), and one high-frequency unit (c). Note the shifted frequency axis in the third unit. The small arrows denote the division between the low- and the middle-frequency range, i.e. 400 Hz, and between the middle- and the high-frequency range, i.e. 900 Hz. The stimulus ensemble consisted in all three examples of short tones with onset intervals of 128 ms (SE III).

except perhaps in one case. One clear BF was found that corresponded to the centre of the frequency range in which saturation occurred. The unit shown had a BF of 1350 Hz and a latency of 10 ms, its STS extended well into the mid-frequency range.

The STSs for three *long-latency units* are shown in Fig. 10. In Fig. 10a a low-frequency unit, BF = 240 Hz, with a latency of 50 ms is shown. The sensitivity extends beyond the frequency range that was investigated. In Fig. 10b a mid-frequency unit, BF = 680 Hz, with a latency of 90 ms is shown. Fig. 10c shows a high-frequency unit, BF = 1760, with a latency at BF of 38 ms but showing considerable longer latencies at frequencies away from BF. Occasionally long-latency units were found which showed a considerable time-jitter in their responses to tones. Such a unit is shown in Fig. 11; a BF is estimated to be 370 Hz; at BF the latency was estimated at 50 ms.

In Fig. 12 a selection of *broadly tuned neurons* is shown. Arrows indicate the separations between the low-, the middle- and the high-frequency ranges. All time axes show a 128 ms range; the frequency axes either run from 125 to 2000 Hz or from 250 to 4000 Hz. After noting the separation mark at 900 Hz it is clear that several units receive input from the amphibian as well as the basilar papillae (Fig. 12b, c, e-h). Others (Fig. 12a) only received input from the amphibian papilla but from a rather wide area which showed large differences in latencies between the low- and the mid-frequency part. Fig. 12g, h are examples of units receiving inputs from the low-, the middle-, as well as the high-frequency range. One unit was found (Fig. 12i) with double tuning in the high-frequency range. Its upper frequency part fell in the short-latency range, the lower-frequency part in the long-latency range.

Binaural interaction

Binaural interaction was determined in 89 units. In 40 (45%) units binaural stimulation had the same effect as contralateral stimulation (37 EO-units) or as ipsilateral stimulation (3 OE-units), while monaural stimulation of the other side had no effect. In 26 units (29%) contralateral as well as ipsilateral stimulation were excitatory (EE-units), although one side sometimes produced much more spikes than

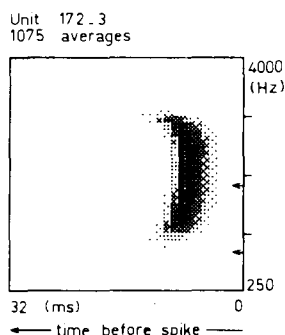


Fig. 9. Spectro-temporal sensitivity of a unit showing a strong saturation. The stimulus ensemble consisted of short-tones with onset intervals of 128 ms (SE III).

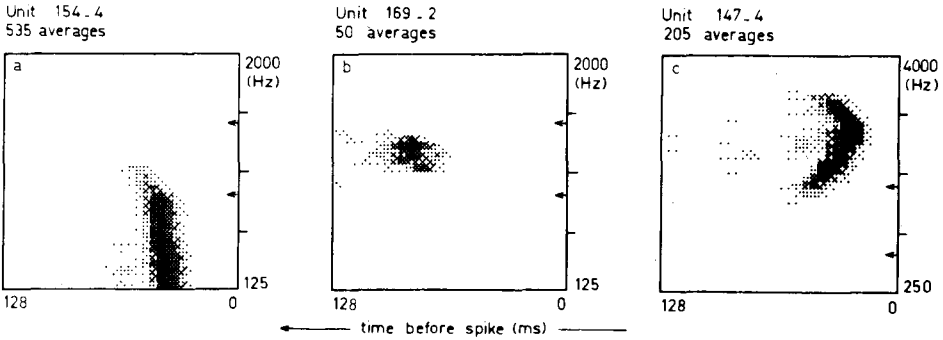


Fig. 10. Spectro-temporal sensitivities of three long-latency units. Note that the time axis differs from the previously shown ones. The stimulus ensemble consisted of long tones with onset intervals of 1 s (SE IV).

the other side. Best frequencies were in most cases very similar and latencies differed for at most 5 ms, the side producing the more spikes showing the shorter latency.

In 23 units (26%) ipsilateral stimulation had an inhibitory influence on contralateral stimulation (EI-units). In most cases the inhibitory effect was so strong that no spectro-temporal sensitivity could be determined under binaural stimulation. In two units where this was nevertheless possible, the latency under binaural stimulation was some 20 ms longer than under contralateral stimulation, while the BFs were similar.

It appeared that EE-units were overrepresented in the population of high-frequency units, while EO- and EI-units were overrepresented among the low- and mid-frequency units (χ^2 -test, $P < 0.05$). EE-units tended to have short latencies, whereas EI-units tended towards longer latencies ($P < 0.1$).

Action potential waveform and neural response properties

It appeared that the type of the spike waveform was not related to the BF of the

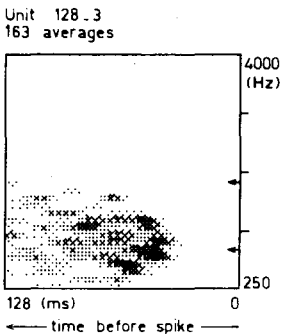


Fig. 11. Spectro-temporal sensitivity of a unit which exhibited very variable intervals between the onset of the tones and the spikes. The stimulus ensemble consisted of tones with onset intervals of 1 s (SE IV).

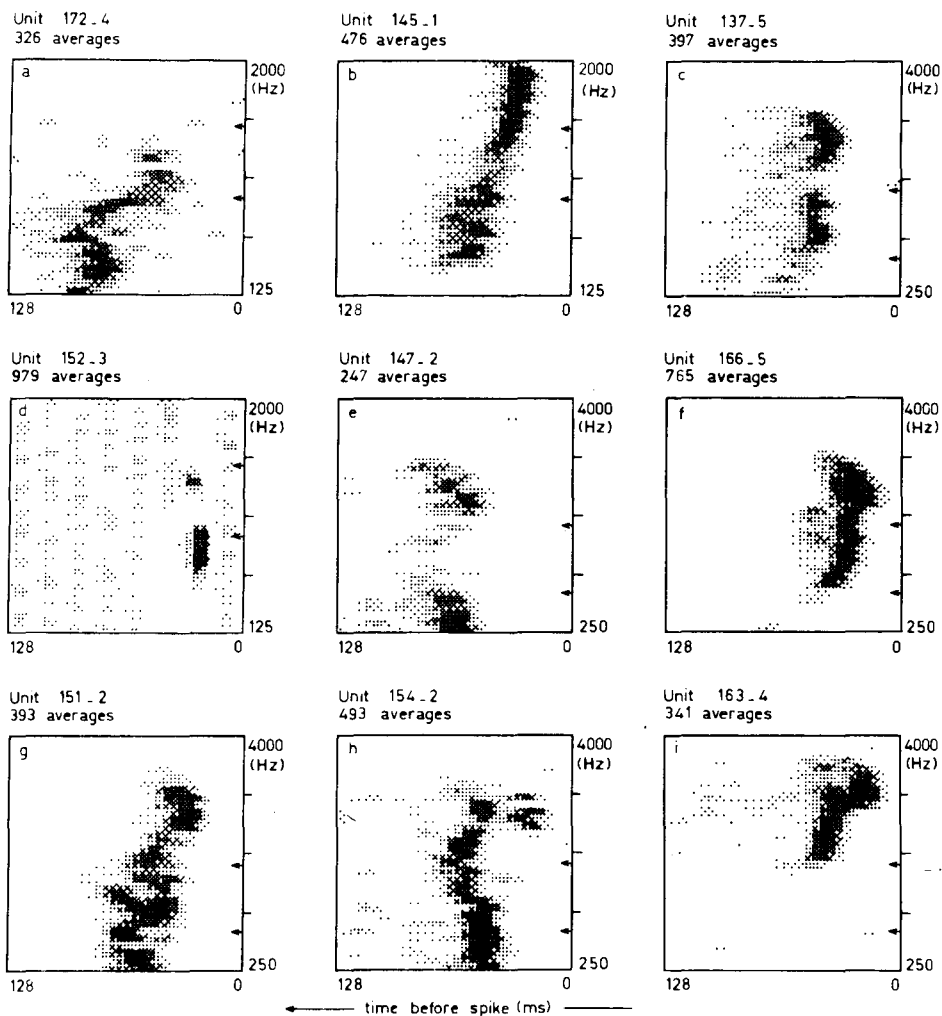


Fig. 12. Spectro-temporal sensitivities of 9 broadly tuned neurons. The SE consisted of long tones with onset intervals of 1 s (SE IV), except in d, where the tones had onset intervals of 16 ms (SE I).

unit. In contrast, a clear correlation with latency was observed (χ^2 -test, $P < 0.005$). All, except one, type-I units had short latencies and type-II units had predominantly long latencies. Spike waveform type III appeared to be equally distributed over short- and long-latency units. Of the six simply tuned neurons with type-IV spike waveform only one had a short latency.

Distribution of neural properties across the torus

The vast majority of the 106 auditory units that could be localised were found

within the classical boundaries [21] of the torus semicircularis. Only 7 were found outside the torus.

No clear *tonotopic organisation* could be found, although high-frequency units showed some preference for the laminar nucleus and the ventral part of the principal nucleus while the dorsal part of the principal nucleus was predominantly sensitive for low frequencies. No significant preference of mid-frequency units for any specific part of the torus was found. Broadly tuned neurons were found in the central part of the torus, especially in the magnocellular nucleus (Fig. 13).

As far as *latency* was concerned, short-latency units were specifically found in the ventral part of the principal nucleus and the transition between the laminar and the principal nucleus. Long-latency units did not show any preference for a particular nucleus (Fig. 14).

As to *binaural interaction*, EO- as well as EI-units were found all over the torus. EE-units were predominantly observed in the ventral part of the torus, especially in the principal nucleus. All three OE-units were located in the laminar nucleus (Fig. 15).

The distribution of *spike waveforms* over the torus is presented in Fig. 16. Units with spike waveform type I were especially found in the ventral part of the principal nucleus. Units with spike waveforms type II were predominantly found in the

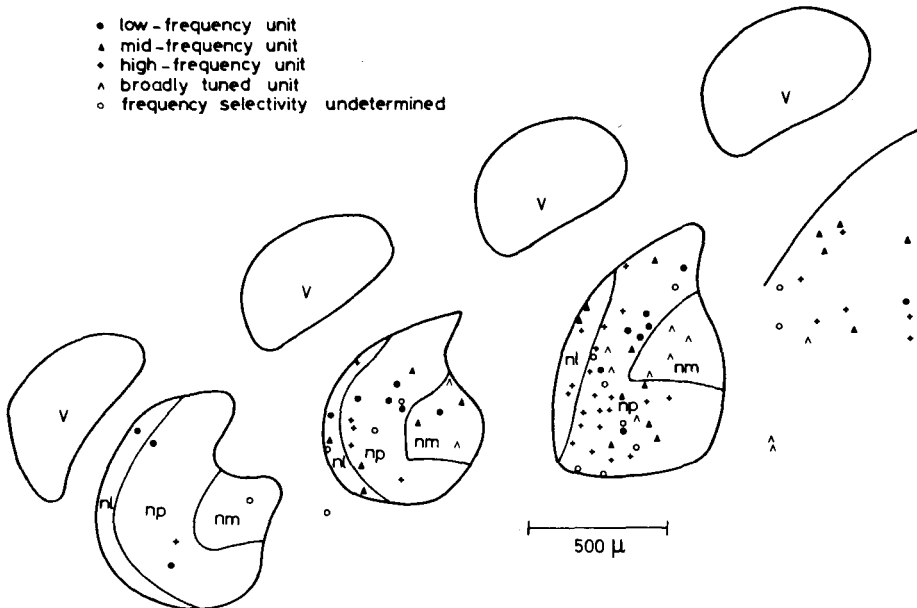


Fig. 13. Distribution of frequency selectivity across the torus semicircularis. The recording sites are indicated in four drawings representing sagittal sections positioned 600, 800, 1000 and 1200 μm laterally from the median. Frontal is to the left, caudal is to the right. V, ventricle; nl, nucleus laminaris; np, nucleus principalis; nm, nucleus magnocellularis.

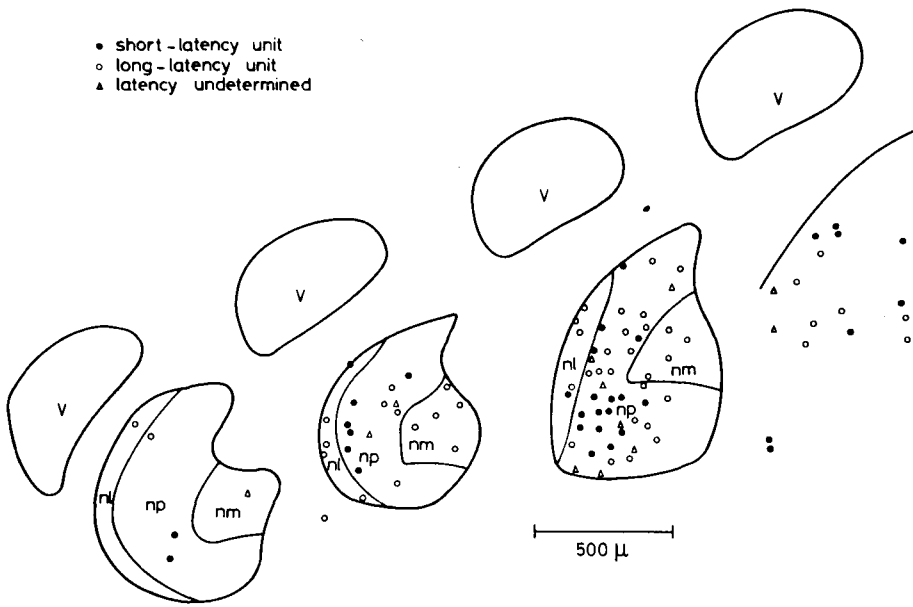


Fig. 14. Distribution of the latencies across the torus.

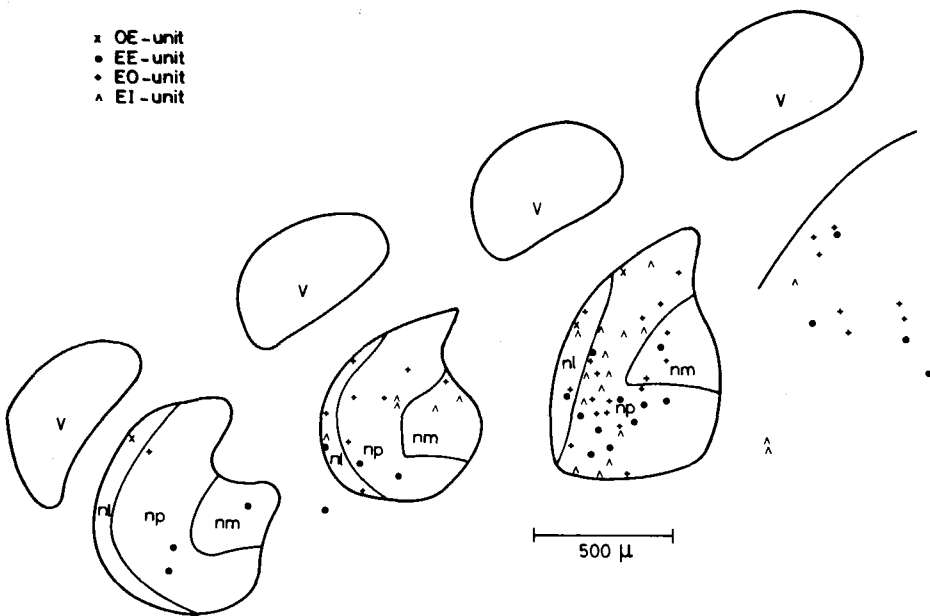


Fig. 15. Distribution of binaural-interaction patterns across the torus.

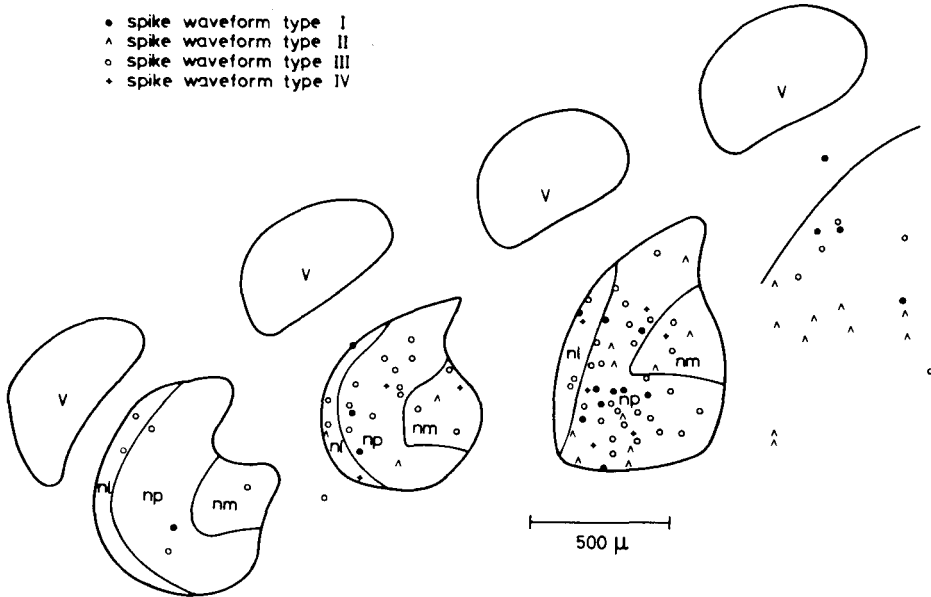


Fig. 16. Distribution of spike-waveform types across the torus.

ventral part of the principal nucleus and in the magnocellular nucleus. Type-III units were found all over the torus, as well as type-IV units.

Correlation between neural properties

The experimental results obtained with tonal stimuli showed various correlations.

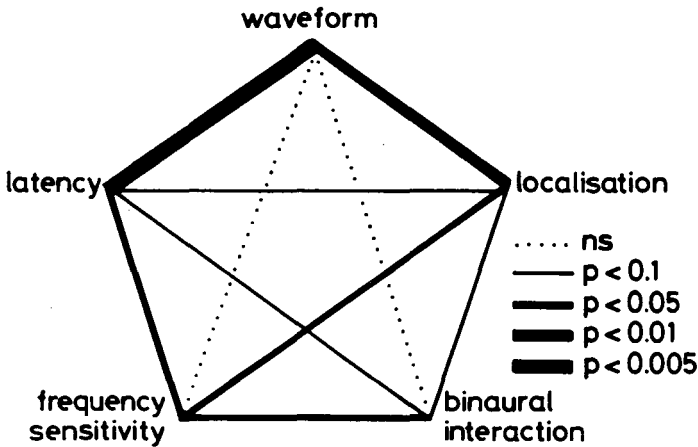


Fig. 17. Diagram summarising the correlation between the various neuronal characteristics. ns, not significant.

As shown in the previous paragraphs, latency seems to be the crucial factor for the correlations. In Fig. 17 the strength of the correlations, on basis of χ^2 -tests, is graphically represented. It appears that latency and waveform type are strongly correlated, as is localisation and waveform. Frequency sensitivity, i.e. basilar papilla input vs. amphibian papilla input, is correlated with latency, localisation, and the type of binaural interaction. Neither correlation was found between waveform type and frequency sensitivity nor with binaural interaction type.

Discussion

The responsiveness of the units

All these experiments took place under light MS-222 anaesthesia. In a previous paper [17] the spectro-temporal characteristics of auditory units were described as determined under stimulation with stationary wide-band noise. It was argued that the light anaesthesia did not affect the response characteristics to noise significantly. As the responsiveness to noise appeared to be strongly correlated with short latencies, the response properties of the short-latency units as determined with tonal SEs were probably not much affected by the anaesthesia either. Just as under noise stimulation the STSs under tonal stimulation did not change very much with changing levels of anaesthesia, although average firing rates could increase significantly when the level of anaesthesia became lower. Furthermore, the measured latencies to tones were clearly correlated to the response times as measured with noise [17], and the BFs under tonal and noise stimulation were very similar. Only one phenomenon that was not encountered under noise stimulation, very regularly occurred under tonal stimulation, viz. saturation. Under tonal stimulation saturation could be very strong, as shown in Fig. 9. Saturation appeared to depend on the level of anaesthesia. In addition under ipsilateral or contralateral stimulation saturation could be quite different. As far as the long-latency units were concerned, the measured STSs did not change much with changing levels of anaesthesia either, although average firing rates often increased when the level of anaesthesia decreased. All these arguments indicate that the anaesthesia increased the threshold of the units without very much affecting the form of the measured STSs.

Sometimes units that first did not exhibit a sustained response to one of the SEs, did so when the level of anaesthesia became lower. This indicates that some of the units from which no sustained response to any of the SEs could be obtained might have exhibited sustained responses when unanaesthetised animals would have been used. In addition, it cannot be excluded that a considerable proportion of auditory units in the midbrain of the grass frog were completely silenced by the anaesthesia. Feng et al. [12] showed that even the responsiveness of auditory-nerve fibres decreased considerably under barbiturate or urethane anaesthesia. Especially fibres from the mid-frequency range of the amphibian papillae were susceptible. This may explain why the proportion of low- and mid-frequency units found in this study was relatively low, about 50%, whereas in immobilised frogs Walkowiak [25] found 78% units with BFs in the low- and mid-frequency range. Another reason for this

discrepancy might be found in the different types of electrodes used by Walkowiak. Walkowiak used glass micropipettes, whereas we used stainless-steel microelectrodes. A third reason for this discrepancy might be a different bias in the electrode penetrations. In this study there is perhaps a bias for lateral penetrations.

In many units silent intervals had to be inserted between the tones in order to obtain a sustained response. To short-latency SEs they fired only at the start after which the firing rate gradually decreased or became zero. This habituation-like behaviour sometimes occurred even when the interval between two successive tones was 1 s. Several mechanisms might explain this. First it is possible that after a tone the probability of occurrence of an action potential first becomes high but then decreases after which the unit remains suppressed for some time. When a tone to which the neuron might otherwise respond is presented during this interval the unit now remains silent. This last tone might then in its turn suppress the firing probability of the unit. These suppressive effects of successive tones might even summate, resulting for the duration of the SE, in ever decreasing firing rates. Only when very long silent intervals are included the suppression will disappear. Second, while tones of some frequencies may activate the units, tones of other frequencies may suppress the neuron. The time course of this suppression may be much longer than that of the activation and also here the suppressive effects may summate. An argument in favour of these mechanisms is that very often units only fired in a sustained way to a SE when long silent intervals were included between the tones.

Frequency sensitivity across the torus semicircularis

A single-unit study in the torus semicircularis of the bullfrog [21] points to the absence of a clear tonotopic organisation. An evoked-potential study in the grass frog [20], however, presents some evidence for a low-frequency sensitivity in more caudal parts gradually changing to a higher-frequency sensitivity in the more frontal regions. Although in our study the recording sites were localised with an accuracy of at best 100 μm and data from many animals were taken together, it is beyond doubt that large parts of the torus are sensitive to more than one of the low-, middle- and high-frequency ranges. This is particularly obvious in the magnocellular nucleus where many units are sensitive to more than one range of frequencies. They receive convergent inputs from different parts of the sensory papillae.

Nevertheless, our results suggest a vague tonotopic organisation at single unit level corroborating the one reported by Pettigrew et al. [20]. The dorsoposterior part of the principal nucleus had a preference for low frequencies, whereas the ventroanterior part appeared to be predominantly sensitive to high frequencies. It is very well possible that a clearer tonotopy in the principal nucleus will be found when a large number of units can be studied in one single animal.

In most cases the BFs of high-frequency units in one frog were very similar. This suggests that the basilar papilla is a rather simple structure tuned to one single frequency [8]. In two cases the results suggest that the tuning of the left basilar papilla can be different from the tuning of the right basilar papilla. In the first frog an EI-unit was recorded with a STS shown in Fig. 12h. The frequency sensitivity of this unit fell below 2000 Hz; the BF for high frequencies was about 1750 Hz. A unit

in the other side of the torus (bilaterally stimulated) had a BF of 2280 Hz. Unfortunately the binaural interaction of this unit was not determined. The second case concerns an EE-unit the STS of which is shown in Fig. 12i. The STS consists of two regions, both in the high-frequency range. It appeared that the range with the higher frequencies was due to ipsilateral stimulation whereas the range with the lower frequencies was due to contralateral stimulation.

Distribution of response latencies

A remarkable finding is the clear division in short- (< 30 ms) and long- (> 30 ms) latency units. These latencies all refer to tones with peak intensities of 89 dB SPL. In other studies latencies were measured at stimulus intensities of a fixed amount of dBs above threshold [14] or at the stimulus intensity that produced the minimal latency [22,25]. As these differ considerably [25] it is not possible to compare our results directly with those of others.

In our previous study using stationary noise as a stimulus [17] it was shown that most short-latency units were responsive in a sustained way to noise stimuli that lasted for several minutes. They were localised in the ventroanterior part of the principal nucleus, more dorsally on the transition region between laminar and the principal nucleus, and in more lateral parts of the auditory midbrain where the distinction between the various nuclei vanished. These regions appear to contain the fibres that enter from the olivary nucleus [24]. These results corroborate those of Potter [21] who mentioned that 5 out of 6 units with latencies less than 20 ms were found in a ventral position in the anterior tips of the laminar and the principal nucleus. He suggested that these units were fibres entering the torus from the olivary nucleus. In addition the range of latencies of these short-latency units (at 89 dB SPL) falls within the range of latencies of units in the olivary nucleus (at 10 dB above threshold) as given by Feng and Capranica [14]. These short-latency units are therefore likely to represent incoming fibres or units that are involved in the first information-processing stages in the torus. Several other arguments favour this conclusion.

First, nearly all units with spike waveform type I had short latencies. It is tempting to assume that these units represent incoming axons, whereas short-latency units with spike waveform type III would represent cell bodies.

Second, another measure for the stage of information processing is provided by the width of the cross-coincidence histogram. This represents the temporal accuracy of firing of a neuron with respect to the stimulus and will be determined by amongst others the number of synapses between the sensory organ and the unit in the torus, and stochastic components in neuronal integration processes. The close agreement between the results based on latency and those on the width of the CCH (Fig. 7) forms another argument in favour of short-latency units representing an early stage of information processing within the torus.

Third, while broad tuning was a regular characteristic of long-latency units, only three short-latency units were broadly tuned. Two of these were found outside the torus in a region between the torus semicircularis and the nucleus isthmus; the third unit (Fig. 12i) showing double tuning in the high-frequency range was another

exceptional unit which was discussed above.

The correlation between latency and frequency sensitivity in the sense that short-latency units were over represented among high-frequency units was also found by Bibikov [5]. The significance of this remains to be investigated.

Binaural interaction

Until recently it was assumed that during monaural stimulation crosstalk to the other ear did not have a significant influence on binaural interaction patterns. This was based on measurements on single fibres of the auditory nerve in conditions where the mouth of the frog was opened [14]. In these conditions the interaural attenuation ranged from 24 to 44 dB. It appeared, however, that crosstalk increased dramatically when the mouth of the animal was closed [9,15] and the attenuation could be as low as 4 dB. This crosstalk is probably due to resonant coupling between the two tympanic membranes by the mouth cavity. It is especially pronounced when stimulation falls in the middle- and the high-frequency range [9]. As our experiments were performed with closed mouths, a monaural stimulus presentation will effectively have stimulated the other ear as well. This effect will especially have been pronounced in the middle- and the high-frequency range and might explain why EE-units are overrepresented in the high-frequency units. It does not, however, explain the low proportion of EE-units in the mid-frequency range. Furthermore, a considerable amount of high-frequency units were classified as EO-units. Here a more complicated mechanism must be present.

In experimental conditions with considerable crosstalk between both ears, an EO-response can most simply be explained by inferring a reciprocal inhibitory influence between both sides. The presence of such an influence has been demonstrated in frogs with open mouths by Feng et al. [13] even at the level of the dorsal nucleus. This mechanism might explain that an EO-unit under closed-mouth conditions will arise as an EI-unit when the mouth is opened. Under monaural stimulation of the contralateral side, the inhibitory influence of the more strongly stimulated contralateral side upon the ipsilateral side prevails, so that crosstalk stimulation of the ipsilateral side cannot exercise its inhibitory influence in the contralateral side. Under monaural stimulation of the ipsilateral side, however, the inhibitory influence of the ipsilateral side upon the contralateral side prevails so that the crosstalk stimulation of the contralateral side is inhibited resulting in no activity whatsoever. Under binaural stimulation a similar situation as under monaural stimulation of the contralateral side will be found. Therefore the units that we classified as EO-units while recorded with mouth closed, will according to this hypothesis turn out to be EI-units when the mouth is opened. This might explain why Feng et al. [14] classified 62% of the auditory units in the torus semicircularis as EI-units in experiments where the frog's mouth was opened. In our experiments 45% of the units were classified as EO-units, and 26% were classified as EI-units. Together this makes up 71%. The EE-units of Feng et al. as well as their EO-units, together 30% in which reciprocal inhibition is absent, will both be classified as EE-units in our study, which closely agrees with the 29% EE-units found by us. Kaulen et al. [19] mentioned 57.2% EO-units, 2.5% OE-units, 20.5% EE-units, 17.9% EI-units and 1.7%

IE-units. These numbers are similar to ours. It is therefore likely that they also experimented under closed-mouth conditions. Although Feng et al. [14] used the green tree frog and Kaulen et al. [19] used an unnamed Chilean frog, the correspondence of the percentages is remarkable. It is suggested that binaural units as classified in mouth-closed situations should not be regarded as one group which differs from monaural units. Functionally, it is preferred to consider EO- and EI-units, under closed-mouth conditions, as one group different from EE-units. The correlation of EE-units with their localisation in the torus and with their frequency selectivity confirms the suggestion that EE-units constitute a functionally different group.

Spike waveform

The spike waveform appeared to be strongly correlated with the latency and the recording site of the unit. This correlation was largely due to the type-I and type-II waveforms. As units with type-I waveforms had short latencies and were predominantly found in those parts of the torus where fibres enter from the olivary nucleus they were associated with spikes produced by myelinated axons. Spike waveform II appeared to be strongly correlated with a long latency and was predominantly found in the ventral part of the torus. The ventral part of the torus is cyto-architecturally characterised by multi-polar cellbodies [6,23], whereas the dorsal part, especially the laminar nucleus is characterised by monopolar cell bodies with one dendritic arborisation directing into the peripheral nucleus. In other parts of the torus monopolar cellbodies are also found, but their direction is less well specified. It is tempting to suggest that units with spike waveform II represent the multipolar neurons [11]. This is favoured by the long latency of these units as well as by finding that about 50% of the broadly tuned neurons had spike waveform II. Their various dendritic arborisations might be the anatomically functional correlate of the inputs from the various frequency ranges they receive. Spike waveform III, then, is the best candidate to represent the unipolar cellbodies or the cellbodies without large dendritic arborisations. So far, however, these suggestions are not more than informed guesses and more detailed anatomical experiments are needed to verify these hypotheses.

Functional and anatomical divisions of the torus semicircularis

The nomenclature for the various parts of the torus semicircularis was adopted from Potter [21]. The proposed major auditory nuclei, the laminar nucleus, the principal nucleus and the magnocellular nucleus, appear to have different neuronal characteristics. The laminar nucleus is the best anatomically defined structure consisting of conspicuous layers of unipolar cellbodies with large dendrites pointing into the principal nucleus. Between these layers run axons from the olivary nucleus. All units in the laminar nucleus are singly tuned, and there are no units with spike waveform II. All 3 units with a binaural interaction pattern type OE were found in the laminar nucleus. The magnocellular nucleus is anatomically characterised by big multipolar cellbodies. Spike waveform type I is lacking and almost all units have long latencies. Many units are broadly tuned, and many have spike waveform II.

The principal nucleus, however, must in our opinion be divided into two functionally different subnuclei. The ventroanterior part appeared to be predominantly sensitive to high frequencies, many units had short latencies and the binaural interaction pattern of many units was of the EE-type, and units with spike waveform type II were regularly met. The dorsoposterior part of the principal nucleus showed a preference for low frequencies. Only few units had short latencies and hardly any binaural-interaction pattern of the EE-type was found. Only occasionally spike waveform type II was met. These arguments warrant a functional subdivision of the principal nucleus.

These considerations, however, can only apply to the more lateral parts of the torus semicircularis, as almost all recordings took place at least 600 μm from the median. Some tracks were made more medially. But even when auditory background activity was recorded, we were unsuccessful in isolating single-unit activity. In many other tracks in the medial parts of the torus responses to substrate vibration were registered but no pure auditory activity could be found. Units responding to substrate vibrations were also found in the more lateral parts of the torus, especially in the most dorsal part of the principal nucleus, which was also mentioned by Potter [21]. These areas either represent a projection from a part of the amphibian papilla that is sensitive to substrate vibrations or represent a projection from other acoustico-vestibular sense organs.

For all our data it has to be kept in mind that the accuracy in localising a recording site was at best 100 μm . In addition, the divisions between the various nuclei of the torus semicircularis are not as clear as the illustrations suggest, and considerable differences between different frogs could be observed. Our data comprise 46 frogs, and if a comparable number of units could be recorded in one frog, the results might be more conclusive. Apart from the laminar nucleus inspection of different kinds of anatomical preparations of the torus semicircularis do not reveal a clearly structured organisation. The correlation found between the various neuronal characteristics and the localisation in the torus despite all these uncertainties, however, do suggest that some organisational plan must exist.

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