

Background firing in the auditory midbrain of the frog



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ABSTRACT

Statistical characteristics of background firing in the midbrain auditory units of grass frog (*Rana t. temporaria*) located in torus semicircular (TS) were investigated. Only about 5% of the cells demonstrated prominent spontaneous firing. For such units the following characteristics were obtained: the distribution of interpulse intervals, the autocorrelation functions (ACF) for the real firing process and for the process with shuffled intervals, the hazard function (HF) and the joint distribution of adjacent interpulse intervals. The burstiness of firing was also estimated. In the absolute majority of the cells, the background firing demonstrated considerable deviation from the renewal process. There was weak but significant positive correlation between adjacent interpulse intervals. The burstiness of firing in the midbrain auditory units was moderate but higher than reported for medullary auditory neurons. The value of burstiness did not decrease after interval shuffling. Along with the reduction in excitability (generalized refractoriness) in many neurons observed post-spike facilitation effects were observed. Comparing background activity in medullary and midbrain nucleus suggests that there is an increase in complexity of the information processing along the auditory pathway.

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Significance statement

In the midbrain auditory center only a few units revealed any firing in the absence of external sounds. I analyzed the statistical properties of background activity in this limited population (less than 10% of the total number of registered cells) and compared these characteristics to the properties of neurons located in the dorsal medulla nucleus of the same species, where the percentage of units with evident background firing exceeded 50%. It was found that in spite of the rarity of spontaneous firing in the midbrain, it is more variable. In some units it tends to form bursts or other specific features that are more typical for units located in higher parts of the auditory system. The results show a significant difference in signal processing on the periphery of the auditory pathway, where the full description of the signal is provided and in the midbrain, where the selection of useful features should be done.

1. Introduction

The sequence of action potentials generated by central nervous system neurons in the absence of controlled stimuli (spontaneous or background activity) is widely used to study neuron parameters,

such as refractoriness (Gaumond et al., 1982) or variability (Lowen and Teich, 1992; Bibikov and Dymov, 2007). Auditory pathway nuclei differ significantly in the percentage of spontaneously active neurons as well as in the mean rate and temporal parameters of firing. In mammals, the highest percentage of spontaneously active units exists in the auditory nerve (Kiang et al., 1965; Manley and Robertson, 1976). In the cochlear nuclei complex the percentage of neurons with evident background activity is generally reduced, although the frequency of background firing in certain cell types may be even higher than at the periphery (Pfeiffer and Kiang, 1965). In anesthetized mammals the sharpest decline in the occurrence of spontaneously active cells takes place in the midbrain auditory centre – inferior colliculus (Mulders and Robertson, 2009). For example, in anesthetized guinea pigs before acoustical trauma, only eight of 266 inferior colliculus cells generated background firing with spike rates greater than 10/s, and in only two cells it exceeded 20/s (Mulders and Robertson, 2009). More spontaneously active neurons are present in decelerated animals (Ramachandran et al., 1999) and in animals studied without any anesthesia (Bock and Webster, 1974). A downward trend in spontaneously active cells along the brainstem auditory pathway is also evident in lower vertebrates, particularly in anurans (Christensen-Dalsgaard et al., 1998; Bibikov and Kalinkina, 1982; Bibikov, 1968).

Temporal features of spontaneous activity in the auditory nerve fibers were initially described as Poisson point process with short dead-time determined by short refractoriness (Kiang et al., 1965;

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Manley and Robertson, 1976). However, current evidence suggests that spontaneous activity is more complex. It has been demonstrated that changes in excitability after spike generation can continue for several tens of milliseconds (Gaumont et al., 1982). A detailed analysis of background nerve firing revealed significant differences from a renewal process. It was determined by accumulated refractoriness for small periods (Gaumont et al., 1982) and chaotic slow changes in excitability for large periods (Lowen and Teich, 1992). Note that the accumulated refractoriness is sufficient to explain the phenomenon of short-term adaptation of the auditory nerve fibers (Bibikov and Ivanitsky, 1985; Bibikov, 2013).

The great majority of cells located in medullar auditory nuclei display prominent background firing. Temporal features of spontaneous activity units located in the mammalian cochlear nucleus were described in an earlier paper (Molnar and Pfeiffer, 1968). Then this classification was repeatedly refined and the relation between the characteristics of the background activity and other properties of cells was defined. Thus, cells with cartwheel dendrites located in the dorsal cochlear nucleus usually generate bursts in their activity. Fusiform cells of the same nucleus are characterized by the ordering (quasiperiodicity) of their firing (Bibikov et al., 2005). The diversity of the background activity was also observed in auditory medullar nuclei of the lower vertebrates, such as tailless amphibians (Bibikov and Ivanitsky, 1992; Bibikov, 1993; Bibikov and Dymov, 2007, 2009). Firing from all auditory nuclei located on the medullar level comes to the main auditory nuclei – inferior colliculus in mammals and TS in frogs. However, in the absence of external sounds, the majority of the neurons located in these midbrain centers are silent. Since the units located in the midbrain auditory centre rarely demonstrate background activity such firing was not studied in detail. Only recently interest in spontaneous activity of the midbrain neurons appeared in connection with the analysis of the origin of tinnitus (phantom sounds). Damage to the inner ear can cause tinnitus in people and an increase in the background activity in the inferior colliculus of experimental animals (Chen and Jastreboff, 1995; Ma et al., 2006; Mulders and Robertson, 2009; Ropp et al., 2014). However, even in these publications the statistical properties of this firing were also not explored in detail, as was noticed in the recent review (Eggermont, 2015): “Except of one study (Ma et al., 2006) none of midbrain studies reported on temporal aspect of spontaneous firing”.

The statistical properties of the background firing in the amphibians auditory midbrain cells remain practically unknown. Our earlier work (Bibikov, 1968) described only qualitative features of the background activity of this structure in the lake frog (*Rana ridibunda*), and the follow-up data were presented only in the form of abstract (Bibikov and Dymov, 2007). In this study I provide a detailed description of the background activity of a relatively small population of auditory midbrain neurons that generate firing activity in the absence of controlled stimuli. The results obtained are compared with the background activity in the dorsal nucleus (Bibikov and Ivanitsky, 1992; Bibikov, 1993; Bibikov and Dymov, 2007, 2009).

The hypothesis was that the differences in the statistical properties of the background activity between medulla and midbrain indicate the transformation of sensory processing in these two levels of the auditory pathway. I believe that these putative differences are present in mammalian auditory systems as well. They may reflect the transition from a detailed description of the temporal flow of the signal to the extraction of its peculiarities and useful features. The experiments are, to the best of my knowledge, among the first to compare background activity in midbrain and medullary auditory centers in the same species.

2. Methods

I used the data obtained in the years 2003–2013 on the common grass frog (*Rana t. temporaria*). More than 200 animals were examined and the whole population of investigated units exceeds 1000. More than 90% of the animals were males. Animal preparation and recording of single unit activity was described previously in detail (Bibikov and Nizamov, 1996; Bibikov and Dymov, 2007, 2009; Bibikov, 2013). Briefly, frogs were anesthetized by hypothermia (Kaplan, 1969; Suckow et al., 1999) and (or) MS222 during surgery (small hole in cranium). During recordings, which began more than an hour after the surgery, the frogs were immobilized by intramuscular injection of alloferine chloride (10 mg/g body weight) and local anesthetic (lidocaine) was applied to the wound sites. Animal body temperature was – 19–21 °C. In this study we followed ethical protocols of animal use in neurophysiological studies (Zimmermann, 1987) and approved by the ethical commission of the institute. Experiments were performed in double-walled sound isolated booth with sound level in the A range (32.5Hz–8,5 kHz) less than 40 dB SPL. Therefore in frequency bands of any single unit of the frog it was less than 20 dB and is considerably below the threshold for all investigated units. The sound was passed from wide-band speaker through the closed tube. It was measured near the tympanum by the condenser microphone.

Spike activity of single neurons was recorded with glass microelectrodes filled with 2.5M solution of NaCl (tips 1–3 mkM, impedance 5–10 mgOm) - Microelectrode was moved dorsoventrally through the tectum opticum to TS by a micromanipulator with a stepping motor (minimal step 4 μm). Entry of the electrode into the TS was detected by an appearance of multiunit activity caused by the auditory search stimulus (100ms duration wide-band noise bursts at the level of 80 dB SPL). In a number of experiments we have confirmed the electrode position histologically. As a rule it was positioned in the central nucleus of the TS.

Characteristic frequencies and thresholds of well-isolated units were determined by manually sweeping the frequency and level of tone bursts. Only cells showing spontaneous firing with the rate exceeding 0.5 spikes/sec were analyzed. Every spontaneous spike was transformed into standard electrical impulse that passed through analog-to-digital converter to a computer for further processing. The duration of recordings was either 500s or 1000s. Only units with spike amplitudes exceeding noise more than five times were included in the study. The shapes of the spikes usually had two or three-phases, which indicates that their origin were TS neurons rather than incoming fibers. During recordings all interspike intervals (ISIs) were stored and ISI histograms were accumulated. The original sequence of intervals was used for calculation of several other numerical parameters and functions. The mean and standard deviation of the ISIs in the whole sequence were calculated and the coefficient of variation (CV) was derived by dividing the standard deviation of the ISI upon mean ISI. Local coefficients of variation (CV₂) as the local measure of spike time irregularity [Ponce-Alvarez et al., 2010] have been also obtained. CV₂ is the sum of all the values of doubled normalized absolute difference of adjacent intervals divided by the total number of intervals. It is calculated using the following equation:

$$CV_2 = \frac{1}{N} \sum_{k=1}^n \frac{2(D_{k+1} - D_k)}{(D_{k+1} + D_k)}$$

where D_k – is k -th interspike interval and N – whole number of interspike intervals. For processes with slow chaotic fluctuations of the firing rate, the CV₂ is usually considerably less than standard coefficient of variation.

Hazard functions (HF) were calculated from ISI distribution as the conditional probability of occurrence of certain interpulse interval in the absence of intervals smaller than specified by:

$$q_n(t) = \frac{p_n}{1 - \sum_{k=1}^{n-1} p_k}$$

where $q_n(t)$ - the conditional probability of the spike generation in the n th interval in the absence of intervals smaller than $n\Delta t$ and p_n - probability of the spike generation in the n th interval (see Gaumont et al., 1982; Bibikov and Dymov, 2007). The presented hazard functions were obtained by dividing conditional probability $q_n(t)$ by the bin duration (0.0005s) and were presented as instantaneous firing rate. The hazard function evaluates the change in excitability following the spike generation in an investigated unit.

The standard histograms of autocorrelation functions of the point process of spontaneous firing with the bin size of 0.5ms were also calculated. I normalized autocorrelation functions (ACF) in the same way by dividing the correlation (from 0.0 to 1.0) in every bin upon the bin duration. The values of correlation functions at 0 delay equal to 2000 (1/0.0005) were omitted. Both histograms of normalized hazard and normalized autocorrelation functions were presented as an instantaneous firing rate and therefore they could easily be compared. In the range of small delays (autocorrelation) and small interspike intervals (hazard function) the temporal courses of these functions are similar for any renewal point processes. Thus the comparison of these functions could be useful for statistical description of the process. With increasing interspike intervals using of hazard functions becomes impractical due to the sharp decrease in the probability of long interspike intervals.

The intercorrelation of the adjacent intervals was determined using a linear regression analysis of two-dimensional distribution of the adjacent (n and $n+1$) intervals also known as the ISI return map. The slope of linear regression in this graph gave us the neighboring ISIs correlation coefficient and reliability of this dependence was calculated. The degree of burstiness in background activity in investigated cells have been estimated by calculating the number of events with the following features: two intervals longer than 1.5 times the average, separated by two or more intervals of less than half of the average. For Poisson point process the percentage of such events, the mean number of spikes in a burst, and the total percentage of spikes included in the bursting activity do not depend upon the mean firing rate and can be calculated from the standard exponential interspike distribution. This calculation shows that corresponding values are on average 1.28%, 2.65 spikes and 3.38%. For the quantitative estimation of burstiness I used the last parameter - the percentage of spikes with short interspike intervals included in the bursts. The original sequence of intervals was also shuffled randomly. Shuffling creates a time series where ISI and CV are identical to those of the original spike train, but the correlation between the intervals is absent. The shuffled sequences were studied using all of the above statistical characteristics. The hazard functions, being rigidly connected with the ISIs, do not change after shuffling. The shuffling also does not affect the steady-state value of the autocorrelation function, which (after our normalization) is always equal to the average spike rate (see Kim et al., 1990). However, the dynamics of the autocorrelation functions, the CV_2 , the burstiness and interdependence between neighboring intervals can be significantly changed. In these cases the recorded background activity was not a renewal point process in which each interpulse interval is independent of its prior history. Numerical calculations were performed by standard statistical methods using Excel and Origin 8. Standard linear regression analysis was used to calculate the relationship between neighboring

intervals in the sequence, as well as between different parameters of background firing.

3. Results

The results presented are based on 82 units of grass frog torus semicircularis, which exhibited evident spontaneous firing. These included cells with constant form and amplitude of spikes, which could be held for 500 or 1,000s without obvious trends. They accounted for about 5% of investigated TS cells. The response to the tonal bursts was detected in 77 of these cells. The characteristic frequencies of these cells ranged from 0.09 kHz to 2.8 kHz, and the thresholds ranged from 24 to 80 dB SPL. The range of these parameters was not statistically different from the data obtained in a larger population of units of the same species (Bibikov and Kalinkina, 1982). Five cells that demonstrated evident spontaneous firing were located in the TS, however, they did not respond to tones. It could be assumed that they probably respond to more complex or more intensive sounds. So I included them in the database. The rate of the background firing in TS units ranged from 0.5 to 22.46 pulses/sec.

In our population of neurons statistically significant correlations between spontaneous rate and characteristic frequency (CF) ($p = 0.26$) or threshold value at a CF ($p = 0.53$) were not observed.

I attempted to classify all investigated neurons with spontaneous activity into three groups based on the shape of the autocorrelation and hazard functions, as it was done in dorsal medullary nucleus of the same species (Bibikov and Dymov, 2007). The total units which could be designated to one of these three groups are illustrated on Fig. 1. In this figure several statistical parameters that characterize the background firing are also shown. The CV and CV_2 values are presented in the right corners of the upper row plots. The correlation coefficients between neighboring intervals (R) and its reliability of dependence (p) are represented in the second row of the chart. Average rates, burstiness values (with ranges obtained for the surrogate sequences with shuffled ISIs) are presented in the third row of the chart. Neurons with interspike intervals near the Poisson distribution which showed constant (within statistical scatter) values of ACF and HF, after comparatively short delay values of 3–8ms, were classified as belonging to the first group. In this group the spike probability was restored to the mean firing rate very soon after a previous spike. Autocorrelation and hazard functions were similar for units in this group (Fig. 1a). The values CV and CV_2 were not far from unity. However, evident positive correlation of neighboring intervals have been observed in the majority of the cells belonging to this group. Burstiness of firing was usually equal or lower than in the Poisson process.

The spontaneous activity of neurons attributed to the second group was characterized by large values of the shortest interspike intervals and a gradual increase of the ACF and the HF to the stationary asymptotic value. Thus the duration of the refractory period in this group was considerably greater than that of neurons of the first group. The neuron illustrated in Fig. 1b was attributed to this group, even though short interspike intervals appeared sporadically in its firing. Meanwhile, autocorrelation and hazard functions values were close to zero up to 20ms delay and increased sharply in the period from 25 to 50 ms, remaining roughly constant after that. Values of CV and CV_2 are both considerably less than unity. The positive correlation of neighboring intervals is even more obvious than in the previous example. The burstiness of firing was considerably lower than it should be for the Poisson point process.

The cells attributed to the third group were characterized by the presence of local maximums both in ACFs and in HFs. These maximums usually followed after absolute refractory periods. In

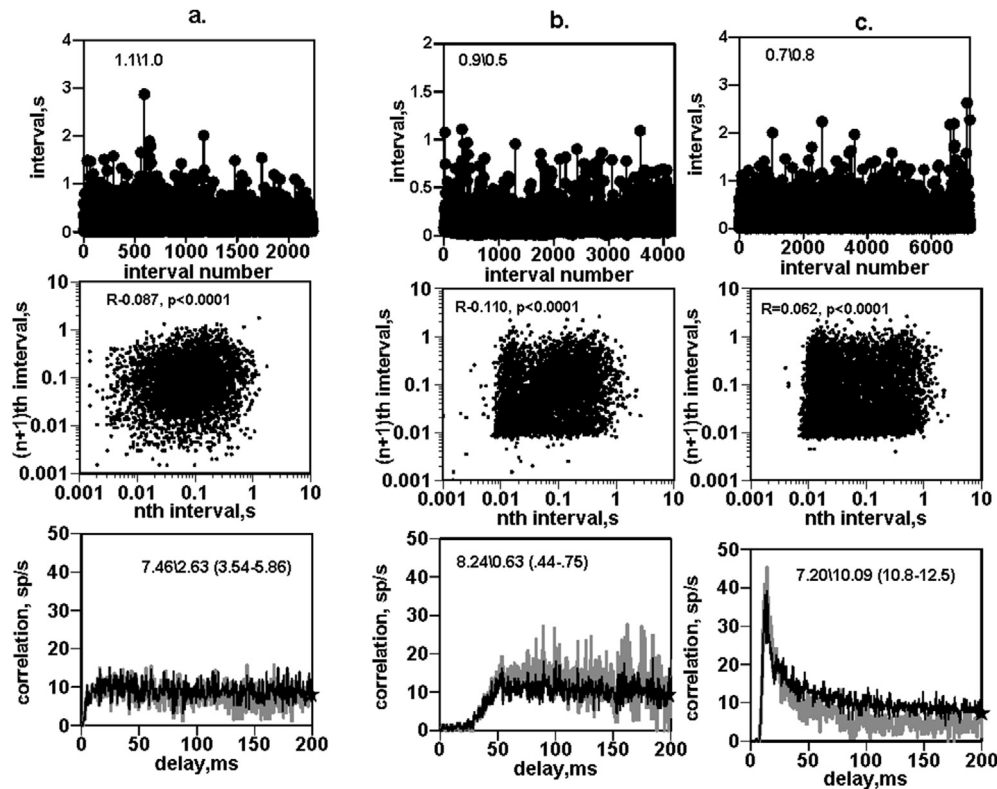


Fig. 1. Basic statistical characteristics of the spontaneous activity in three torus semicircularis neurons. a) neuron 021101 (CF = 0.61 kHz, N = 3731) with near Poisson firing, b) neuron 070201 (CF = 0.27 kHz, N = 4132) that demonstrated considerable decrease in excitability for a long time after spike generation; c) neuron 062107 (CF = 0.28 kHz, N = 7204) with a tendency to enhanced burstiness. In the top row the successive values of interpulse intervals are presented. In the upper part of the plots CV and (through slash) CV₂ values are given. In the middle row the joint histograms of the adjusting interspike intervals are presented. The correlation coefficient (R) and level of significance (p) of the dependencies of the adjusting ISIs are given in the upper part of the plot. In the lower row correlation functions (black) and hazard functions (grey) are presented in terms of instantaneous rate. The mean firing rate and (through slash) burstiness are given in the upper part of the plot. In parentheses the spreads of burstiness values for 15 shuffled sequences are given. The stars show mean firing rate.

the unit illustrated on Fig. 1c, these functions rose sharply in a very short interval from 7th to 12th ms and then declined to the steady-state level exponentially with time constants around 12ms.

For this unit the values of CV and CV₂ were rather low, and the interdependence between neighboring intervals was not evident. This neuron had a relatively high level of burstiness. This parameter was three times higher than the value expected for Poisson random process. Interestingly, the burstiness only increased after shuffling of interspike intervals (Fig. 1c).

The asymptotic values of the ACF and HF for the investigated units were compared. It is well known that ACFs converge to the asymptote, corresponding to the average firing rate (see star's marks in Fig. 1, lower row). The asymptotic level of the HF corresponds to the firing rate, which would have existed if post-spike changes in excitability were absent. In accordance with this, the final values of the HF are above the ones for ACFs for neurons with severe refractoriness (Fig. 1b) and are below the ones for ACFs for neurons with increased excitability after the spike (Fig. 1c).

However, the classification that was used for the medullary auditory units is not sufficient to systematize the population of TS units with spontaneous activity. Even the neurons illustrated in Fig. 1 do not fully correspond to the categories that were introduced for the dorsal medullary nucleus (Bibikov and Dymov, 2007). For example, the neuron with severe refractoriness was characterized by a noticeable number of very short ISIs (Fig. 1b), and the neuron with increased excitability after a spike had a rather long refractoriness and low values of CV and CV₂ (Fig. 1c). Even the units illustrated in Fig. 1a had some peculiarities. The burstiness of impulsation was lower than it is typical for the Poisson process and increased after

interval shuffling. Probably this cell had some faint hidden periodicity in its spontaneous firing. Moreover, approximately half of TS cells with evident spontaneous firing could hardly be included in one of these groups, because they were characterized by specific features of the autocorrelation functions. Consequently, the strict classification of investigated neurons into several clearly defined groups or clusters seems impractical.

Six examples of units which couldn't be attributed to these groups are illustrated in Fig. 2 and Fig. 3. In several units very short intervals were strongly overrepresented. Fig. 2 shows three examples of such units. The firing of the cell (Fig. 2a) resembled the Poisson impulsation with strong overrepresentation of very short (2.0–4.0ms) intervals. Since such intervals were comparatively rare, the burstiness of the firing exceeded the value typical for Poisson process only slightly and the burstiness did not change considerably after interval shuffling. This is due to the fact that inburst interval value (<0.5 of mean value - = 107ms) already included many rather long intervals (see Fig. 2b). Two-dimensional distribution demonstrates a strong deficit of interspike intervals in the range 0.01–0.05c (Fig. 2b).

Even more complex properties of firing could be observed in the units illustrated on Fig. 2c. At least three evident maximums with very different arguments (2–3ms, 5–20ms and 200–1000ms) clearly separated by sharp minima could be observed in the distribution. On the autocorrelation function two first maxima are clearly visible. The burstiness of the firing in the cell is very prominent both in original sequence and after intervals shuffling.

Fig. 3 shows three examples of spontaneous activity of the cells exhibiting some signs of order or periodicity in their

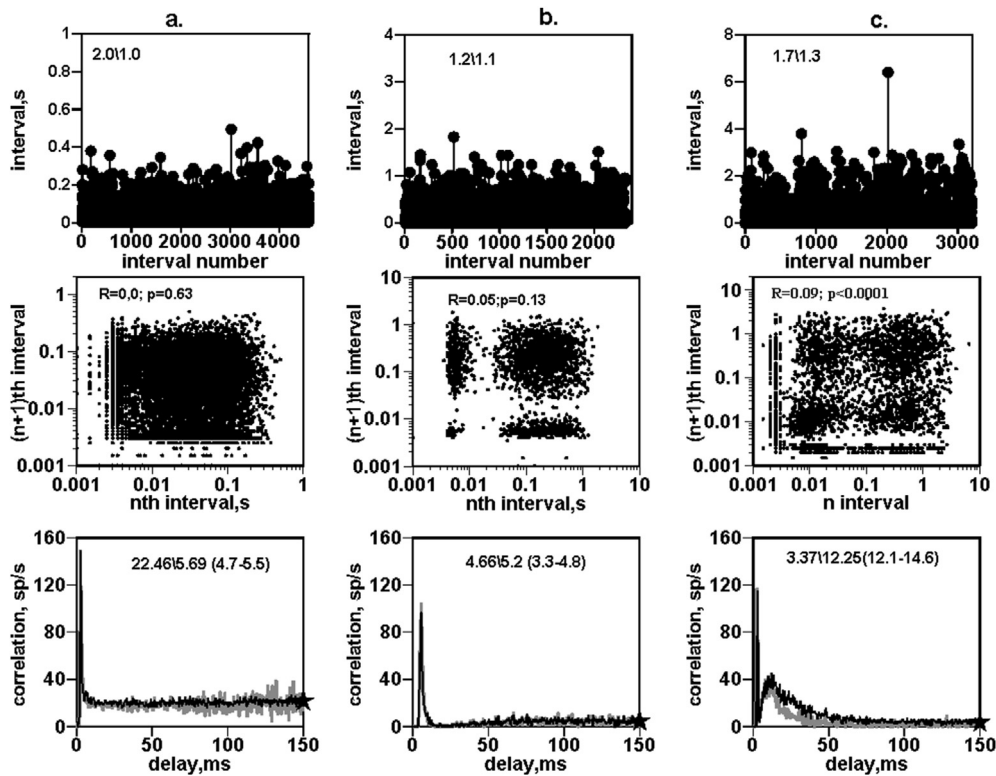


Fig. 2. Basic statistical characteristics of the spontaneous activity in the three torus semicircularis neurons with prevalence of very short interspike intervals. a) neuron 021103 (CF = 0.73 kHz, N = 10221) with Poisson-like interspike distribution except for the prevalence of very short intervals, b) neuron 090913 (CF = 0.21 kHz, N = 2328) in which after the period with the prevalence of very short intervals goes the period of reduced spike probability, followed by restoration of average firing rate; c) neuron 000206 (CF = 0.41 kHz, N = 3265) with complex dynamics of post-spike changes of the autocorrelation function and the hazard function. All designations are as in Fig. 1.

firing. The correlation functions, illustrated in the bottom rows on Fig. 3a and b, are rather similar to those observed for Poisson distribution of interspike intervals. Such parameters as CV, CV₂ and even burstiness also lay in the range that is typical for a Poisson process. However, several maximums with constant internodes period (Fig. 3a) as well as the one evident maximum in the range 100–150ms (Fig. 3b) in the HF and ACF indicate that the nature of the background activity in these cells is more complex. Complex forms of these functions with some ordering of firing can also be observed for the unit illustrated on Fig. 3c. For all the cells illustrated on Fig. 3, the positive correlations between neighboring intervals are evident.

The relationships between different statistical parameters of the spontaneous activity for investigated units have been explored. There were no evident correlations between parameters of spontaneous activity (mean rate, CV, CV₂, burstiness) and characteristic frequency and the threshold of evoked response (for all these relationships $p > 0.1$). I could not find any evidence for correlation of the firing rate with CV, CV₂ and burstiness (for all these relationships $p > 0.4$).

However, the correlation between parameters, characterizing temporal features of the firing was evident. Lower row of Fig. 4 illustrates that the CV was positively correlated with the CV₂ ($R = 0.51$, $N = 82$, $p < 0.0001$), with burstiness of firing ($R = 0.37$, $N = 82$, $p = 0.0002$). The CV₂ was positively correlated with burstiness ($R = 0.55$, $N = 82$, $p < 0.0001$).

The analysis of two-dimensional distributions of adjacent intervals showed that in the majority of cases there is a significant positive correlation between adjacent intervals. The correlation coefficient of this dependence was rather low (< 0.1). However, due to the relatively large sample of spikes in each recording, in 59 out of 82 neurons this dependence was quite reliable ($p < 0.005$).

Therefore, for the vast majority of neurons there is a trend whereby short intervals are followed by short ones, and long intervals are followed by long ones. This result indirectly suggests that there are slow chaotic changes in the firing rate.

However, among investigated units, three neurons demonstrated negative correlation of short adjacent intervals. One of these units is illustrated on the Fig. 3c. In this cell the negative correlation was quite evident for the intervals shorter than 100 ms ($R = -0.11$; $N = 1512$; $p < 0.0001$) in spite of positive correlation over the whole range of ISIs ($R = 0.05$; $N = 5075$; $p < 0.001$).

4. Discussion

The TS of frogs, as well as inferior colliculus of mammals, are the midbrain components of the auditory pathway. It integrates virtually all inputs from the auditory brainstem. It is natural to assume that at this level of the auditory system single neurons performed not a simple description of the temporary course of the signal, but they provide an extraction of its characteristic features. Interestingly, on this level of the auditory pathway the sharp decrease in the background firing is observed in virtually all taxonomic groups of vertebrates. The results reported for *Rana temporaria* are consistent with the data we reported on this species earlier and with the data obtained on other American and European frog species (Bibikov, 1968; Gooler and Feng, 1992; Goense and Feng, 2005). Just recently only a minority of spontaneously active neurons were observed in TS of awaked *Rana pipiens* (Ponnath et al., 2013; Ponnath and Farris, 2014). Interestingly, more spontaneous activity was recorded in six specimens of grass frog caught in the amplexus (Walkowiak, 1980). Since our recordings were carried out in the resting season, the difference can be explained by the state of the animal's hormonal systems (Arch and Narins, 2009).

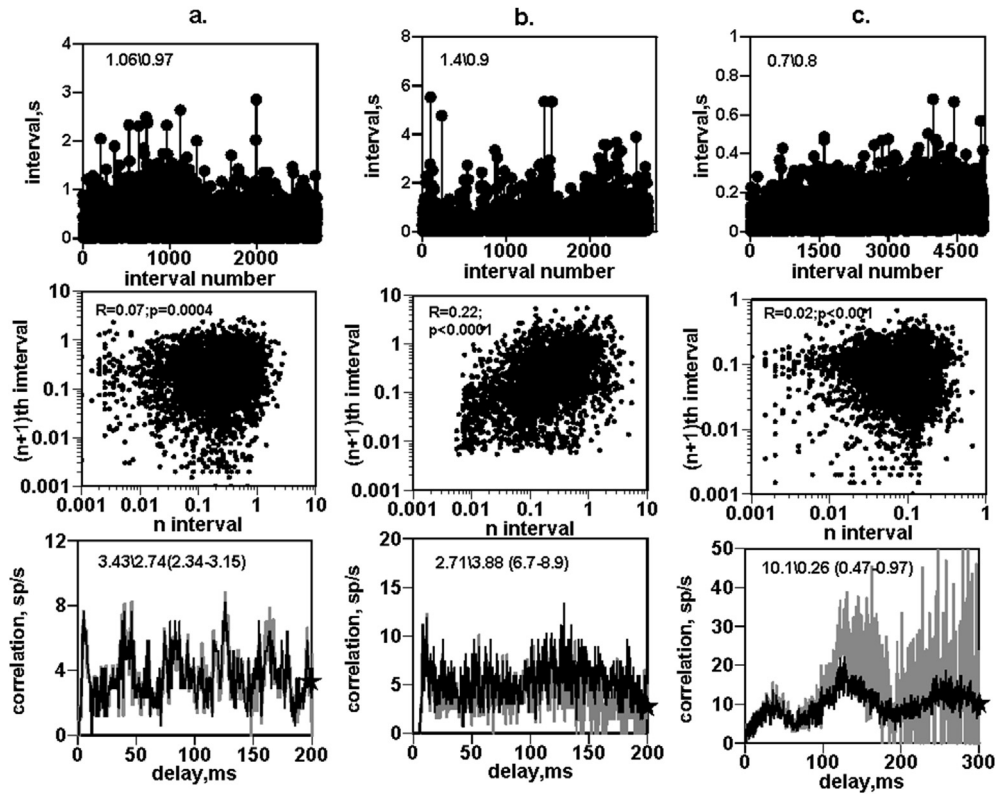


Fig. 3. Basic statistical characteristics of the spontaneous activity in three torus semicircularis neurons with some periodicity in the firing. a) – neuron 0404009 (CF = 0.37 kHz, N = 2664) with several peaks both in autocorrelation and hazard functions, b) – neuron 0401001 (no resp., N = 2706) with near Poisson firing except for the presence of the maximum in the range of 100–150ms; c) – neuron 000903 (CF = 0.79 kHz, N = 5076) with complex dynamics of post-spike changes of the autocorrelation function and the hazard function with a few maximums around 120ms and 170ms. All designations are as in Fig. 1.

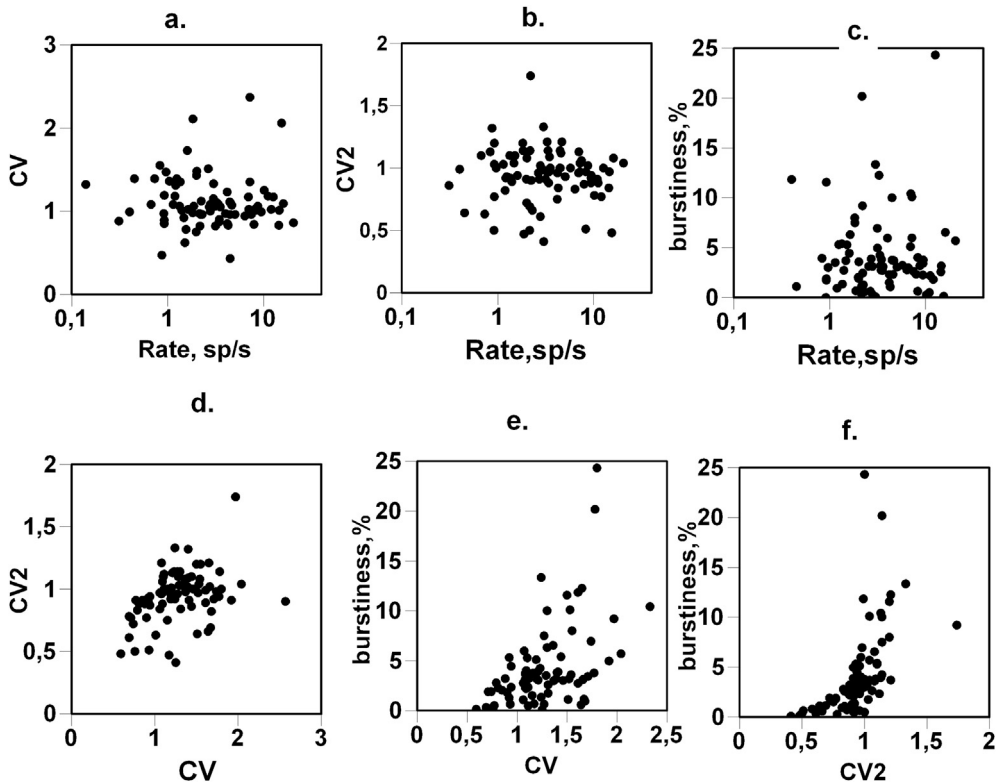


Fig. 4. Relationship between parameters of the spontaneous activity in torus semicircularis units a) CV versus firing rate; b) CV₂ versus firing rate; c) burstiness versus firing rate; d) CV₂ versus CV; e) burstiness versus CV; f) burstiness versus CV₂.

A comparatively high proportion of spontaneous activity, with a rate more than 1 spike per 10 s, was also reported in low-frequency and vibration sensitive neurons (Christensen-Dalsgaard and Jorgensen, 1989). The effectiveness of sound-proofing in very low-frequency range can affect these results.

In mammals the percentage of spontaneously active cells of the midbrain auditory centre is normally very low. It increases sharply immediately after any violations in the cochlea of the inner ear that causes a temporary or permanent increase of the audibility threshold (Chen and Jastreboff, 1995; Ma et al., 2006; Mulders and Robertson, 2009). The temporal characteristics of spontaneous activity in vertebrate's midbrain auditory center are not properly characterized. It represents an evident contrast to medullary auditory centers where this problem was investigated both in mammals (Pfeifer and Kiang, 1965) and amphibians (Bibikov and Dymov, 2007, 2009). Evidently it was partly determined by the rarity of the spontaneous firing in the auditory midbrain.

Meanwhile, an abrupt decrease in spontaneous activity in this structure likely indicates the fundamental difference in the nature of signal coding between medullary nuclei and inferior colliculus. It was very likely that this difference would also be reflected in the statistical properties of neurons' spontaneous activity.

I compared the peculiarities of spontaneous activity in the medulla (Bibikov and Dymov, 2007, 2009) and in the midbrain (this study) without references to the difference in the relative number of such neurons in these auditory regions. The data showed that in the grass frog, midbrain auditory units demonstrate a considerable variety of temporal characteristics in their spontaneous firing. Although for many spontaneously active TS cells there are some counterparts among the dorsal nucleus neurons, the evident differences between them should be noted even in these cases. For example, the combination of severe refractoriness with prolonged evident facilitation, like in the cell illustrated in Fig. 1c, was not observed in the dorsal nucleus neurons. Also, in the dorsal nucleus of the medulla we have not seen cells with high and narrow autocorrelation peaks with a very short delay. Such peaks were observed in six midbrain units, three of which are presented in Fig. 2.

Moreover, the units with spontaneous firing showing considerable deviations from Poisson statistics are more typical for TS as opposed to dorsal nucleus. For example, in the TS some units demonstrate virtual absence of short ISIs (Figs. 1b and 3c). It could be treated either as long and pronounced refractoriness or as evidence of long-time integration constant in the frames of the "integrate and fire" model. We have already described units with long-time integration constants in TS of several anuran species (Bibikov and Gorodetskaiya, 1981).

In a few neurons located in dorsal medullary nucleus there was a negative correlation between short neighboring intervals (Bibikov and Dymov, 2007). In three TS units this effect was also quite evident. We have already mentioned that negative correlation between short neighboring intervals indicates an existence of the accumulated refractoriness. Such accumulated refractoriness can adjust unit's threshold to the level of the incoming signal, leading to differential sensitivity of the cells to small changes in signal amplitude (Bibikov and Ivanitsky, 1985). The role of refractoriness and negative correlation of adjacent intervals in improving differential sensitivity was also demonstrated in electro-sensory systems of fishes (Ratnam and Nelson, 2000) and in other objects (see Avila-Akerberg and Chacron, 2011).

However for the whole ISIs the sign of correlation coefficients between neighboring ISIs was positive for all investigated cells. For 59 out of 82 neurons the positive correlation was quite reliable ($p < 0.001$).

For 72 of 82 units the value of ISIs standard deviation (CV) exceeded the value of local variation (CV_2). In many cases this difference was quite evident (see values in upper right corner of upper

rows in Fig. 2a, c, 3b). For Poisson distribution these values should be equal. Both of these features (positive correlation of neighboring interval and prevalence CV over CV_2) could be explained by the existence of slow chaotic changes in neuron excitability. The long-term positive correlation of ISIs is typical for fractal point processes. It might be determined either by the internal neuron properties or by the properties of the network it embedded in.

Overall, our data support the conclusion that the background activity in the majority of auditory neurons is not a renewal process either for short or for long ISIs (Lowen and Teich, 1992). On short intervals the negative correlation of adjacent intervals can be observed, for large intervals this correlation is usually positive.

In many spontaneously active toral units, both in ACFs and in HF's the local maximums immediately following the refractory periods are observed (Fig. 2a, b, c). The amplitude and width of these maximums were quite different among units. A question about the mechanisms underlying these primary maxima is related to the problem of burst generation in different neurons of the auditory pathway. There is evidence of burstiness and prevalence of short interspike intervals even in auditory nerve of low vertebrates, such as Caiman crocodiles (Klinke and Pause, 1980, their Fig. 2B) and in a few lizard species (Koppl and Manley, 1990, Fig. 7B).

Neurons with the local maximum of the autocorrelation function and some burstiness in their firing were described earlier in the dorsal medullary nucleus of the grass frog (Bibikov and Dymov, 2007; Bibikov, 2013). However, in this nucleus, the percentage of interspike intervals included in bursts (calculated by the above-mentioned method) doesn't reach a value of six. Meanwhile, in some TS neurons the percentage of interspike intervals included in bursts reached 12% (Fig. 2c). Such high burstiness is more typical for higher nuclei of the brain (Bibikov and Pigarev, 2015).

It can be suggested that there is a general tendency for the increase of burstiness in spontaneous firing along the auditory pathway from peripheral to central regions of the brain. For mammalian auditory nerve fibers the concentration of short intervals has been reported only in the prenatal and early postnatal states (Rubel and Fritzsche, 2002). In adult animals it can be observed exclusively in pathological conditions (Lieberman and Kiang, 1978). Studies performed on ventral cochlear nucleus neurons also suggest a very small probability of bursts in spontaneous firing (Molnar and Pfeiffer, 1968), although again this activity is common among neurons of different parts of the cochlear nucleus during early stages of ontogeny (Brugge and O'Connor, 1984). In adult mammals, the burstiness was typical only for the so-called cartwheel inhibitory cells located in the dorsal cochlear nucleus (Manis et al., 1994). Bursts generated by these cells usually consisted of a few spikes whose amplitude decreased towards the end of the burst. We observed such activity neither in the dorsal nucleus nor in the TS of the grass frog. However, some midbrain cells of the frog demonstrated a high proportion of pairs of spikes with very short ISI (Fig. 2b). One of the possible mechanisms of the burst generation could be the existence of dendritic spikes (Stuart and Spruston, 2015). In spite of a shortage of studies on the temporal parameters of spontaneous activity in the auditory units from superior olive up to medial geniculate body, it could be hypothesized that there is a systematic increase of proportion of units with the burstiness in their spontaneous activity along the auditory pathway. An especially considerable increase of burstiness was observed in the auditory cortex. I suggest that the increase of burstiness in neuronal firing along the sensory pathway is a general tendency. The same trend has been observed in the visual system (Saleh and Teich, 1985; Teich et al., 1997).

Burst patterns in the TS unit's can play an important role in animal's behavior. Interestingly, bursts in sensory units solely determine cricket escape behavior caused by sound (Marsat and Pollack, 2006).

The relationship between burstiness and other characteristics of spontaneous firing was rarely subjected to special analysis even for the mammalian auditory system. High values of burstiness for neurons with high dispersion of interspike intervals (measured by CV and CV₂) observed in this paper (Fig. 4b) are quite expected. High variation indicates the relative increase in the proportion of extremely large and extremely small values of ISI.

The origin of bursting activity and prevalence of short interspike intervals requires its own explanation. One possible explanation is that such neurons have short refractoriness and low-frequency variation of membrane potential. Another hypothesis connects the bursting activity with neuronal plasticity. It is based on a simple neuron model with Hebbian synaptic plasticity (Grigor'ev and Bibikov, 2010). The model has plenty of inputs (spines) each of which is initially "tuned" to a certain temporal interspike interval. The synaptic weight of spines tuned to the small intervals automatically grows due to the fact that the number of short intervals is relatively high in any noisy point process. For example, in the Poisson process the relative number of intervals exponentially decreases with increasing intervals.

Special consideration is needed for cells with periodic background firing. The tendency for periodicity in firing was observed in small subpopulations of cells both in the dorsal nucleus and in the TS (Fig. 3). Out of 82 units, nine neurons showed some periodicity in their firing. For four of them, it is likely that this activity was the response to a periodic vibration. For two of them, the preferable intervals were near 20ms and for two - near 10ms. For all of them, spike amplitudes were large enough to completely eliminate the possibility of artifacts related to electrical interference. High sensitivity to vibration in some toral units is well known (Narins and Lewis, 1984; Christensen-Dalsgaard et al., 1989). The only reasonably stable source appears to be building vibration on the power supply frequency of 50 Hz (in Russia). Vibratory sensitive units investigated in US laboratories tended to be phase locked to 60 Hz – the power supply signal in the USA (Lewis et al., 1985). We have observed several units in dorsal medullary nucleus that have demonstrated "spontaneous" firing with evident 50 Hz periodicity (Bibikov and Dymov, 2007). All of them had low characteristic frequency and probably were connected with saccular afferents (Comer and Grobstein, 1981).

In our current study five units with rhythmic activity had a preferred period which was not confined to multiples of 50 Hz. In two of them, preferred intervals of background firing correlated with best modulation period of their responses to amplitude-modulated tones (data not shown). The same effect have been observed in a medullary unit of *Rana ridibunda* earlier (Bibikov, 1993). The search for auditory neurons with internal periodicity in their firing system was carried out for a couple of decades in different animals. For a long time the best candidates for the role of such cells were neurons with chopper discharge located in ventral cochlear nucleus (Langner, 1988). However, a few studies showed that in the vast majority of choppers, the constancy of ISIs is determined exclusively by exceptionally strong though short refractoriness. This conclusion can be made because of the absence of oscillations in the distribution of first spike latencies (Bibikov et al., 2002). Interestingly, there are internal periodic oscillations not associated with the refractory effects in a few neurons located in the anuran auditory pathway (Fig. 3, also Bibikov, 1993; Bibikov and Dymov, 2007). Therefore, I believe that in TS of grass frogs there is a small population of units with internal periodicity in their background activity.

Our data demonstrate that despite low percentage of spontaneously active neurons in the auditory center of the midbrain, the diversity of statistical characteristics of their impulsion is greater than the diversity of statistical characteristics of medullary auditory neurons. This relatively small population of cells not only includes three groups with spontaneous activity similar to the

background activity of the dorsal medullary nucleus neurons, but also contains units with new properties. I have observed the cells with a pronounced tendency to demonstrate a prevalence of very small interpulse intervals (Fig. 2a) and the cells with evident burstiness (Figs. 2c and 3a). Almost all joint distributions of the adjacent intervals demonstrate a clear tendency to a positive relationship, which can be explained by the presence of slow chaotic changes in excitability. Therefore, for such units, the process of spontaneous firing can't be considered a renewal point process. Moreover, in a few units, sophisticated features of the autocorrelation functions and the joint distributions of adjacent intervals (see. Figs. 2a,b and 3c) resemble the properties of spontaneous activity recorded in the central auditory system of mammals (Bibikov and Pigarev, 2015).

Therefore, presented data adds new evidence in favor of the existence of common characteristics of auditory processing in brainstem auditory pathways in anurans and mammals.

Both in mammals (Kiang et al., 1965) and in anurans (Christensen-Dalsgaard et al., 1998) peripheral auditory units perform a thorough description of the temporal course of the sound. For this purpose the background activity could be quite meaningful because it helps to reproduce fine temporal variation of the signal. The midbrain auditory neurons do not simply reproduce the temporal course of the sound, but extract some specific peculiarities of the amplitude dynamics. For these purposes the presence of spontaneous activity can be an obstacle.

Conflict of interest

The author declares no competing financial interests.

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Abbreviations

- TS: Torus semicircularis
 ACF: function of autocorrelation
 HF: hazard function
 CV: coefficient of variation
 CV₂: local coefficient of variation
 CF: characteristic frequency