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Analysis of the time evolution of auditory steady-state responses (ASSR) recorded in rats V.2

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Habituation of Auditory Steady State Responses Evoked by Amplitude-Modulated Acoustic Signals in Rats

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We use this protocol and it's working

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Abstract

Auditory steady-state responses (ASSRs) are brain oscillations locked to the periodic properties of acoustic stimuli. Audiological tests based on the acquisition of ASSR are useful for estimating the hearing sensitivity, mainly because multiple hearing frequencies can be simultaneously assessed, and the auditory response can be objectively detected using statistical tests. Typically, the extraction of the auditory response from the measured signal essentially relies on averaging epochs of the EEG, time-locked to the stimulus. Such a manipulation assumes that the auditory response is steady over time and that averaging increases the signal-to-noise ratio of the measurement. Since the time-domain averaging of epochs within a recording does not allow to discriminate between methodological and physiological related variations in the amplitude of the ASSR, we designed a protocol for analyzing the dynamics of the auditory response during the acquisition procedure. The protocol allows us to compute the ASSR amplitude at a given time window without being compromised by those computed in the preceding EEG segments. In other words, the ASSR amplitudes are extracted from individual epochs, without those epochs being time-domain averaged with the preceding EEG segments. As a result, adaptation and other non-stationary behaviors of the ASSR can be studied.

Attachments

PDF

ASSR_adaptation_2015..

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486KB

Guidelines

The study must be performed under approval of the local Animal Research and Ethics Committee. Especifically, this research was performed under approval of the Animal Research and Ethics Committee of the Cuban Neuroscience Center, conformed to the guidelines of the National Center for Animal Breeding of Cuba.

Safety warnings

Animals should be manipulated following standard safety proceedings. Standard safety procedures should be considered for the manipulation of disposable needles and syringes. National and local electrical safety regulations must be followed.

Before start

Care, feeding, breeding, and maintenance of animals should follow standard local guidelines. Animals should be housed in a standard bio-clean animal room under a 12-h light-dark cycle at 22-24°C, with free access to food and tap water.

Preparation

1 Animals are anesthetized with ketamine (75.0 mg/kg, ip) and diazepam (5.0 mg/kg, ip).

2 Supplemental doses of anesthesia are administered during the experiment at a level sufficient to maintain the animal in an areflexic state.

3 Atropine sulfate (0.06 mg/kg; im) are administered to decrease the mucosal secretions.

4 Body temperature is maintained at 37.0±0.1°C by a body temperature control system.

5 Animal sacrifice is not required. Animals are returned to the colony after recovering from anesthesia.

Acoustic stimulation and EEG recording

6 Acoustic stimuli are presented monaurally, via an ER 3A Etymotic Research insert earphone.

7 Custom-fitted ear molds are used to replace the original foam to permit the earphone to be coupled to the rat's ear.

8 The calibration of the stimulation system is needed. In our experiments, the acoustic levels are referred to a Brüel & Kjær artificial ear (type 4152). Calibration is performed using a Brüel & Kjær 2250 sound level meter (Brüel & Kjær 4144 microphone).

9 Acoustic stimuli can be generated using standard hardware/software. In our experiments, continuous tones of 8 kHz sinusoidally-modulated in amplitude (95% depth) at 115 Hz are generated using the ASSR software module of the AUDIX system (Havana, Cuba). Stimulus intensity is fixed at 50 dB SPL.

10 Electrophysiological responses are recorded differentially using stainless-steel needle electrodes inserted subdermally (vertex positive; neck negative; thorax reference).

11 Recordings are amplified with gain 1.2×104 and band-pass filtered –cutoff frequencies of 10 and 300 Hz.

12 Output of the filter is digitized at 16 bits of resolution and sampled at 920 Hz.

13 Segments with peaks of electrical oscillations exceeding 50 mV are rejected online.

14 Data acquisition continues until completing 60 artifact-free epochs of 4.45 s (4096 time-points each).

00:10:00 time between consecutive recordings

Thirty recordings are acquired from each animal.

Data processing

15 In our experiment, processing of the data is performed using in-house Matlab codes (MathWorks, USA). Please, contact the authors if you are interested in using the codes.

16 The 60 sequential epochs of the 30 recordings are re-arranged offline into a data matrix of 30 rows and 60 columns -one matrix per animal.

17 Optionally, the dataset can be modified to reduce the influence of the noise on the computation of the auditory response.

Note

For example, epochs can be weighted by dividing each voltage sample by the variance in amplitude of the epoch they belong to, so that variance can be used as a measure of variability and weighting factor. Pertinently, the weights need to be normalized by their average across all epochs in order to make the ASSR amplitudes comparable with those resulting from other procedures.

18 Epochs in the data matrices are column-wise averaged. In other worlds, for each time window, the epoch of 30 recordings were averaged to reduce EEG background noise and detect the ASSR amplitude.

19 The amplitude of the ASSR is computed once for each group of epochs, at the end of the averaging, using the fast Fourier transform (FFT). In our experiments, we use an FFT length of 4096 time-points, which correspond to the length of an epoch (4.45 s).Since we sample the EEG at 920 Hz, the frequency resolution of the FFT was 0.22 Hz. A windowing technique is not implemented.

20 The amplitude of the ASSR is defined as the spectral amplitude obtained at 115 Hz (frequency at which the acoustic stimulus is modulated in amplitude). The amplitude of

the 30 spectral components at each side of the frequency of the response are vector averaged to calculate the residual noise level (RNL).

21 The ASSR amplitudes are compared with the corresponding RNL using the Hotelling's T2 multivariate test implemented in the AUDIX system, which considers both the amplitude and phase of the oscillations. The statistical test was applied once, at the end of the averaging.

22 To describe the time evolution of the ASSR, the ASSR amplitudes are plotted as a function of time. Negative exponential functions are fitted to the time courses (requiring r^2>0.85, and p<0.05 to be consider as a valid fitting).

Expected result

When an adaptive behavior is detected, the adaptation index (P_{adapt}) of the response is calculated using the equation:

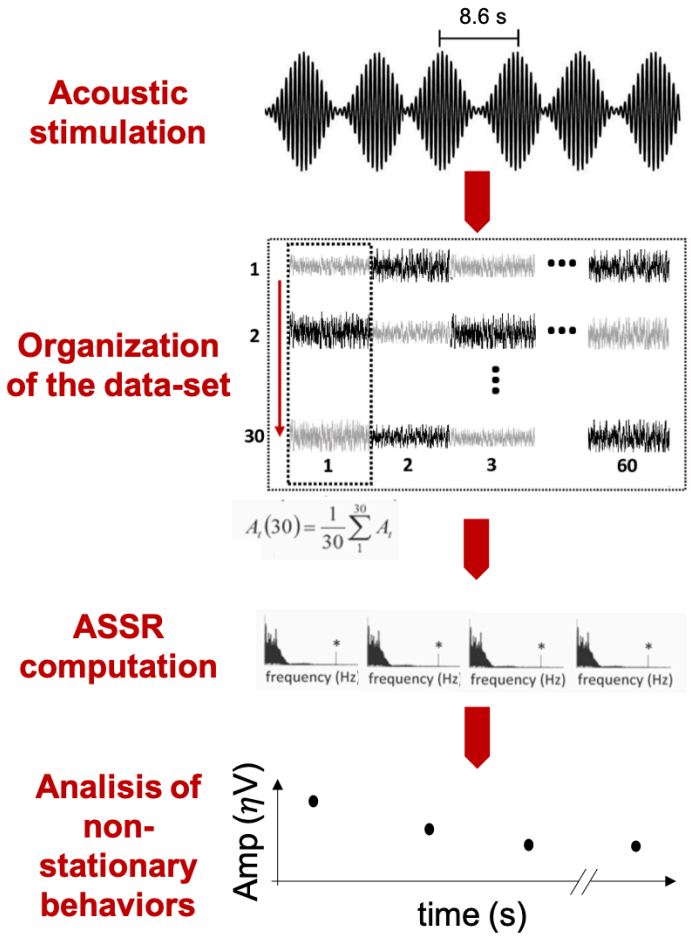
$P_{adapt} = 100(Amp_{max} - Amp_{adapt})/Amp_{max}$

Where Amp_{max} represents the maximum amplitude of the fitted curve and Amp_{adapt} represents its asymptotic value (defined as the amplitude estimated when the recording length was three times the time constant of the fitted exponential function)

23 Statistical tests should be applied as needed. In our analysis, One-way ANOVAs (p<0.05) and the corresponding post-hoc analyses (Tukey test, p<0.05) are performed to analyze the stability of the ASSR amplitude and the RNL within a given number of epochs.

Summary

24 A summary of the protocol is presented below. From top to bottom, they are represented i) the acoustic stimuli modulated in amplitude at 115 Hz; ii) the organized data-set (matrix with 60 columns and 30 rows, where rows are individual recordings and columns are the EEG epochs); iii) the spectral analysis of the averaged measured, and iv) the graphical representation of the ASSR dynamics.


Acoustic stimulation
8.6 s
Organization of the data-set
1
2
30
1
2
3
60
$A_t(30) = \frac{1}{30}\sum_{1}^{30} A_t$
ASSR computation
frequency (Hz)
frequency (Hz)
frequency (Hz)
frequency (Hz)
Analisis of non-stationary behaviors
Amp (ηV)
time (s)