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SPECTROTEMPORAL RECEPTIVE FIELDS DURING SPINDLING AND NON-SPINDLING EPOCHS IN CAT PRIMARY AUDITORY CORTEX

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Abstract—It was often thought that synchronized rhythmic epochs of spindle waves disconnect thalamo-cortical system from incoming sensory signals. The present study addresses this issue by simultaneous extracellular action potential and local field potential (LFP) recordings from primary auditory cortex of ketamine-anesthetized cats during spindling activity. We compared cortical spectrotemporal receptive fields (STRF) obtained during spindling and non-spindling epochs. The basic spectro-temporal parameters of "spindling" and "non-spindling" STRFs were similar. However, the peak-firing rate at the best frequency was significantly enhanced during spindling epochs. This enhancement was mainly caused by the increased probability of a stimulus to evoke spikes (effectiveness of stimuli) during spindling as compared with non-spindling epochs. Augmented LFPs associated with effective stimuli and increased single-unit pair correlations during spindling epochs suggested higher synchrony of thalamo-cortical inputs during spindling that resulted in increased effectiveness of stimuli presented during spindling activity. The neuronal firing rate, both stimulus-driven and spontaneous, was higher during spindling as compared with non-spindling epochs. Overall, our results suggests that thalamic cells during spindling respond to incoming stimuli-related inputs and, moreover, cause more powerful stimulus-related or spontaneous activation of the cortex. **© 2008 IBRO. Published by Elsevier Ltd. All rights reserved.**

Key words: local field potentials, multi-unit, ketamine, spindling, spectro-temporal receptive fields, auditory cortex.

During sleep and under anesthesia the thalamo-cortical network is hyperpolarized relative to the waking state and is characterized by global synchronous oscillations in different frequency bands. It is frequently expressed that the thalamo-cortical system is functionally disconnected from the outside world during rhythmic global thalamo-cortical oscillations (Llinas and Steriade, 2006), or that the information from specific thalamic relay inputs might be significantly modified (Sherman and Guillery, 2004). Sherman and colleagues (Lu et al., 1992; Guido et al., 1992) demonstrated, by *in vivo* intracellular recording (cats, halothane anesthesia, paralyzed preparation) that hyperpolarized lateral geniculate nucleus (LGN) relay cells, when in arrhythmic burst mode, do respond to visual stimulation with low-threshold calcium spikes accompanied by bursts of action potentials. Thus, in LGN, stimulus-related EPSPs can switch low-threshold Ca^{2+} channels from the deinactivated to the activated state and lead to bursts of conventional action potentials, which in turn can activate cortical cells. In other words, the arrhythmic thalamic burst mode does not disconnect the thalamocortical system from ongoing sensory input. However, it is still unclear, whether thalamic relay neurons can show stimulus-related activity when they are involved in global synchronized rhythmic behavior and especially during spindle oscillations. Synchronized or desynchronized behavior of neuronal populations is reflected in the corresponding synchronized or desynchronized pattern of electroencephalography (EEG) and local field potentials (LFP). Indeed, it is synchronized population thalamic activity that conveys rhythmic action potentials to the cortical cells and produces EPSPs, which are reflected in the EEG or LFP spindle-related pattern (Steriade et al., 1993a). Recordings from different (non-auditory) thalamic nuclei in immobilized rats clearly demonstrated a global neuronal involvement of particular thalamic nuclei in spindle oscillations and the presence of thalamic multi-unit synchrony with spindle-related EEG (Buzsáki, 1991).

So far, a systematic analysis of thalamo-cortical information processing during synchronized epochs of spindle oscillations has not been done. Spectrotemporal receptive fields (STRFs) have been routinely recorded in ketamine-anesthetized cat cortex both for high-density (aggregate tone-pip rate 120 Hz) and low-density (aggregate tone-pip rate 20 Hz) multi-frequency sound and during strong or barely detectable spindle waves (Valentine and Eggermont, 2004; Eggermont, 2006). We recently showed that high-density multi-frequency sound suppresses spindling activity during ketamine anesthesia in cat primary auditory cortex and that cortical STRFs were evoked by stimulation (Britvina and Eggermont, 2008). However, it was not investigated if these STRFs were only based on firings during non-spindling epochs or that stimuli-related cortical firing also can be evoked during synchronized epochs of spindle waves. The present study, using simultaneously recorded LFPs and extracellular multi-unit recording from the primary auditory cortex of ketamine-anesthetized cats during prominent spindling activity, addresses this issue. We examined the STRFs to low-density stimuli based on spiking

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Abbreviations: AAF, anterior auditory field; AI, primary auditory cortex; BF, best frequency; BOLD, blood oxygenation level-dependent; CF, characteristic frequency; EEG, electroencephalography; LFP, local field potential; LGN, lateral geniculate nucleus; Npeak, peak value; PSTH, post-stimulus time histogram; STRF, spectrotemporal receptive field.

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recorded during spindling epochs where thalamic neurons are presumably in rhythmic burst mode, and the interleaving non-spindling epochs where thalamic neurons are presumably in tonic-firing mode (Steriade and McCarley, 2005). Our results demonstrate that STRFs of primary auditory cortical neurons are very similar during both spindling and non-spindling epochs, but that the peak neuronal response is enhanced during these spindling epochs.

EXPERIMENTAL PROCEDURES

Animal preparation

All animals were deeply anesthetized with the administration of 25 mg/kg of ketamine hydrochloride and 20 mg/kg of sodium pentobarbital, injected intramuscularly. A mixture of 0.2 ml of acepromazine (0.25 mg/ml) and 0.8 ml of atropine methyl nitrate (25 mg/ml) was administered s.c. at approximately 0.25 ml/kg body weight. Lidocaine (20 mg/ml) was injected s.c. prior to incision. The tissue overlying the right temporal lobe was removed and the dura was resected to expose the area bounded by anterior and posterior ectosylvian sulci. The cat was then secured with one screw cemented on the head without any other restraint. The wound margins were infused every 2 h with lidocaine, and additional acepromazine/atropine mixture was administered every 2 h. The ketamine dose to maintain a state of areflexive anesthesia was on average 9.8 mg/kg/h (range 6.4–12.8 mg/kg/h).

The care and the use of animals reported in this study were approved (#BI 2001-021) and reviewed on a yearly basis by the Life and Environmental Sciences Animal Care Committee of the University of Calgary. All animals were maintained and handled according to the guidelines set by the Canadian Council of Animal Care. All experiments conformed to international guidelines on the ethical use of animals. All efforts were made to minimize the number of animals used and their suffering.

Acoustic stimulus presentation

Stimuli were generated in MATLAB® and transferred to the DSP boards of a TDT-2 (Tucker Davis Technologies, Alachua, FLA, USA) sound delivery system. Acoustic stimuli were presented in an anechoic room from a speaker system (Fostex RM765 Boonton, NJ, USA) in combination with a Realistic Super-Tweeter that produced a flat spectrum (±5 dB) up to 40 kHz measured at the cat's head) placed approximately 30° from the midline into the contralateral field, about 50 cm from the cat's left ear. Calibration and monitoring of the sound field were accomplished with a condenser microphone (Bruel & Kjaer 4134) placed above the animal's head, facing the speaker and a measuring amplifier (Bruel & Kjaer 2636). Prior to acute recordings peripheral hearing sensitivity was determined using auditory brainstem response (ABR) thresholds (details in Noreña et al., 2003).

The multi-frequency stimuli consisted of randomly presented gamma-tone pips, equally spaced on a logarithmic scale. The envelope of the gamma tones is given by:

$$\gamma(t)=(t/4)^2\exp(-t/4),$$

with t in ms. The duration of the gamma tones at half peak amplitude was 15 ms, and the envelope was truncated at 50 ms, where the amplitude is down by 64 dB compared with its peak value (Npeak). Here, tone pips for each of 81 frequencies in five octaves were randomly presented according to a Poisson process (Eggermont, 2006), with similar average rate but different realization for each frequency. Each tone pip frequency was presented at a rate of 0.25 Hz so that the aggregate tone-pip rate was 20 Hz.

Recording procedure

Two arrays of eight electrodes (Frederic Haer Corporation, Bowdoin, ME, USA) each with impedances between 1 and 2 MΩ were used. The electrodes were arranged in a 4×2 configuration with inter-electrode distance within rows and columns equal to 0.5 mm. Each electrode array was oriented such that most electrodes were touching the cortical surface and then were manually and independently advanced using a Narishige M101 hydraulic microdrive Narishige (East Meadow, NY, USA) (one drive for each array). The depth of recording was between 700 and 1200 μm, and thus the electrodes were likely in deep layer III or layer IV. The LFP and spike signals were amplified 10,000 times using a TDT-system 3 (Pentusa) data acquisition system with filter cutoff frequencies set at 2–5000 Hz. The LFPs were obtained by low-pass filtering at 40 Hz and sampled at 610 Hz, whereas the spikes were obtained by high-pass filtering at 300 Hz and sampled at 12.5 kHz.

Data analysis

STRF. The STRF was determined by constructing frequency-specific post-stimulus time histograms (PSTHs) for each of the preceding gamma tones in a 100 ms window. For that purpose, each spike elicited was plotted several times in the appropriate frequency bins and in the 100 ms time window after the onset of each of the preceding gamma tones (since spike latency is a priori unknown). If the gamma tones have no effect on a spontaneously firing neuron, the entire matrix of 81 frequency bins by 100 (1 ms duration) time bins will be filled uniformly. If certain frequencies consistently produce excitation in a certain latency window then this part of the frequency-time plane would receive more hits. In case certain frequencies produce consistently lower activity than average this would be interpreted as inhibition (Valentine and Eggermont, 2004; Tomita and Eggermont, 2005). The STRFs were smoothed using a 5×5 bin window.

Cortical area boundaries. Cortical area boundaries were assessed on basis of a reversal of the characteristic frequency (CF) gradient in the tonotopic map and along the electrode array, minimum latency values, the shape of the STRF, and the Npeak of the cross-correlation coefficient for recordings straddling boundaries. For delineating the border between primary auditory cortex (AI) and anterior auditory field (AAF), we first of all used the sign and/or reversal of the gradient of CF along the electrode array with distance in the anterior direction (Noreña and Eggermont, 2005). The general shorter minimum latency in AAF compared with AI, and particularly the much higher frequency-tuning curve bandwidth at 20 dB above threshold in AAF (Eggermont, 1998) were important as well. For the distinction between AI and posterior auditory field (PAF) or potentially the posterior ectosylvian gyrus (EP) we used mainly latency, which was at least 20 ms larger in non-AI areas. In addition, the sudden drop in peak cross-correlation coefficient across area boundaries under spontaneous firing conditions (Eggermont, 2000) was a highly consistent indicator. The data presented here are all based on recordings from AI.

Construction of STRFs for spindling and non-spindling epochs. We separated the 900 s duration LFP recordings into epochs of spindle and non-spindle activity by discrete windowed Fourier transform (DWFT) analysis with a time window of 500 ms and discrete time steps of 50 ms. At each step, a moving average of the power spectrum with averaging interval of 1 Hz and using a Hanning window was calculated. The time intervals for spindling (or non-spindling) activity were defined as intervals where the LFP power within the 7–14 Hz frequency band in the dynamic spectrum was above (or below) a certain threshold level related to the background noise (Fig. 1). This separation was done for the LFPs recorded from one electrode in the array as there was little difference in the LFP amplitudes recorded at individual electrodes for