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The 5–12 Hz oscillations in the barrel cortex of awake rats – Sustained attention during behavioral idling?

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ABSTRACT

Objective: 5–12 Hz oscillations, observed in cortical LFP of awake rats during quiet immobility, were proposed to be either (i) epileptic events or (ii) physiological alpha-like oscillations, manifesting an idling state of the cortex. We aimed to test this controversy.
Methods: We recorded LFP from the barrel cortex of awake Wistar rats, while applying weak tactile (whisker) and stronger arousing (electrical) stimuli.
Results: We observed a mean effect of desynchronization of the 5–12 Hz rhythm by the weak tactile stimulation. Arousal reduced the incidence of the 5–12 Hz oscillations and increased the desynchronizing power of tactile stimuli.
Conclusions: Oscillations that can be disrupted by weak, purely tactile stimulation, and whose incidence is reduced by increased arousal, should be interpreted as a physiological phenomenon typical for behavioral idling while the cerebral cortex maintains sensory sensitivity.
Significance: Our results contradict the view that the 5–12 Hz oscillatory activity, often observed in fronto-parietal cortical regions of Wistar rats, represents epileptic discharges. Rather, this activity provides a model for studying the physiology of alpha/mu oscillations.

1. Introduction

Neocortical field potential oscillation in an approximate frequency range of 5–14 Hz is a widely observed and clinically significant phenomenon investigated in numerous mammalian species (for review, see Buzsáki, 2006). In rats of various laboratory strains recurrent episodes of high amplitude oscillations in the range of 5–12 Hz can often be recorded from the neocortex of awake, quiescent animals, but the functional interpretation of this phenomenon, since one of the earliest descriptions in hooded rats by Vanderwolf (1975; "7–9 Hz spindles"), remains a matter of controversy. Observed mostly in fronto-parietal regions of the cortex, and usually accompanied by behavioral arrest and fast vibrissal movements (so called "whisker twitching"), these oscillations are similar to discharges observed in rodent genetic models of the absence seizures (WAG/Rij and GAERS rat strains, Vergnes et al., 1982; van Luijtelaar and Coenen, 1986). These features, together with a decreased prominence of the 5–12 Hz rhythm after administration of ethosuximide, a drug applied in petit mal epilepsies, are a rationale for the proposed epileptic nature of this rhythm (Shaw, 2004).

Alternatively, the oscillations may be regarded as a spontaneously enhanced rodent analog of the sensorimotor mu rhythm. The major argument in support of the physiological nature of the 5–12 Hz rhythm is its desynchronization by sensory stimuli (event-related desynchronization, ERD) of different modalities. However, the weakness of this argument may lie in the strength of the stimuli causing desynchronization in the relevant studies: uncontrolled (Vanderwolf, 1975; Marescaux and Vergnes, 1995) or explicitly salient, and thus arousing, stimuli in form of electrical stimulation of the skin (Shaw, 2004), or tactile stimuli associated with a liquid reward (Wiest and Nicolelis, 2003). In such situations the desynchronizing effect could result from an increase in arousal accompanying the sensory stimulation. In this case, the ERD would not sufficiently argue against an epileptic nature of the 5–12 Hz rhythm, since a high level of arousal, or electrical stimulation of the vagal or trigeminal nerves are known to decrease the incidence of absence seizures in humans (DeGiorgio et al., 2006) and pentylenetetrazole-induced seizures in rats (Fanselow et al., 2000).

Even when regarded as physiological, the rodent 5–12 Hz rhythm can be interpreted diversely: as a sign of a resting state of the cerebral cortex (Semba et al., 1980) or as an expression of sensory hypersensitivity optimized for detecting weak sensory signals, which is suggested by thalamic unit responses to sensory stimulation during the oscillatory episodes (Fanselow et al., 2001). Such a "detection mode" in the operation of a sensory system was proposed to reflect

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a "hyper-alert", aroused state of an animal, advantageous in potentially dangerous situations. Its attentive character seemingly contrasts with inattentiveness usually associated with "idling" synchronization in the alpha band (Fontanini and Katz, 2005).

It is important to determine whether the 5–12 Hz oscillatory episodes observed so often in awake rats of various non-epileptic strains represent pathological discharges or physiological mu-like rhythm, as they are encountered probably by most researchers recording from awake rats, i.e. do they signal increased seizure susceptibility that appears in the "normal" laboratory rats colonies due to inbreeding, lack of natural selection, etc., or rather offer a model for studying physiological features of alpha/mu activity?

In order to shed more light on the interpretation of the 5–12 Hz rhythm we recorded local field potentials (LFP) from the barrel cortex of awake rats and analyzed the effects of two kinds of somatosensory stimuli. One of them was a non-salient, slight vibrissal deflection, to which the animals were very well habituated (i.e. a non-arousing, purely somatosensory stimulus). The other was an arousing, electrical stimulation of the skin of rat's ear (i.e. a somatosensory stimulus with an arousing component). This gave us an opportunity to observe how the 5–12 Hz oscillations in awake, undrugged animals are affected by different levels of arousal and, which we find more pertinent, by purely tactile (whisker) non-salient stimuli that are not expected to affect the epileptic discharges (Marescaux and Vergnes, 1995).

2. Methods

2.1. Experimental procedures

The data were obtained from five male Wistar rats, weighing 300–400 g, in the chronic experimental paradigm used in our laboratory (Wróbel et al., 1998; Kublik, 2004; Jakuczun et al., 2005). All experimental procedures followed the 86/609/EEC directive and were accepted by the First Warsaw Local Ethical Commission for Animal Experimentation.

Initially, for 2–3 weeks, the animals were handled and accustomed to a restraining hammock in the experimental room. Then, under deep chloral hydrate anesthesia (intraperitoneal injections of 3.6% solution, 10 ml per 1 kg of body weight), surgery was performed during which 1–6 electrodes (of 0.025 mm insulated tungsten wire, with tips conically sharpened, ~150 kΩ impedance at 1 kHz) were implanted into the barrel cortex of each animal at one or two locations, with tips targeted at various cortical layers, contralaterally to the planned whisker stimulations. Several small (ø 1–1.4 mm) stainless steel anchoring screws were inserted into the skull bone. One of them, placed in the frontal bone, served as recording reference. The screws, electrodes, connectors and a head restraining bolt were secured on the skull with dental acrylic. After the surgery, and during the few days of recovery, the rats received analgesic drugs (Paracetamol, 500 mg per 100 ml of drinking water, or subcutaneous 0.1 ml injections of Rimadyl) and antibiotics (subcutaneous 0.1 ml injections of Baytril).

After the recovery period, during the first recording session we verified electrophysiologically the electrodes' location: we recorded potentials evoked by stimulating each vibrissa (evoked potentials, EPs) and compared their amplitudes. The whisker stimulations consisted of a very small (~0.1 mm) and short (1 ms) down-and-up movement of a piezoelectric slab glued to the whisker about 15–20 mm from the snout. For further experimental manipulations and analysis we chose the whisker either giving the most prominent response measured at the first negative wave observed in the infragranular cortical layers (in case of two rats), or a group of vibrissae glued together, instead of a single whisker, was stimulated (in case of three rats). Then, the animals were subjected to 4–8 habituation sessions (1 h per day) during which they were accustomed to the control conditions of the experiment, i.e. they were suspended in a hammock, head immobilized with the restraining bolt, and subjected to tactile whisker(s) stimulations (100–120 per session), with a pseudo-random interval of 10–45 s. The habituation period ended when the rat would not display signs of stress or visible shudder reactions to the whisker stimulation during the session; however, at no time did the rats fall asleep (at all times they kept their eyes open and the recorded LFP showed no increased slow wave (<4 Hz) activity).

The actual experimental session (the results of which are presented herein) followed the habituation sessions and consisted of two parts: (i) a "control phase" with 30–60 whisker stimulations applied in conditions identical as in the habituation sessions, and (ii) a subsequent "arousal phase", where the whisker stimulations were followed (with 300–500 ms delay) by an arousing electrical stimulation (0.03–0.07 mA, 3 ms square pulse, 1 s train, 50 Hz) applied onto the skin of the rat's ear. The intensity of the stimulation was adjusted so as to evoke a shudder of the rat's body and a twitch of the ear but no vocalization or struggle against the immobilization. Due to latent inhibition instigated by our habituation paradigm, no conditioning was expected or observed during the experimental session.

Throughout the experimental session analog local field potential (LFP) signals from the electrodes were recorded continuously (with a 0.1–5000 Hz bandpass filter, 1000× amplification) on a VHS tape recorder (RACAL V-store, RACAL Recorders Ltd., United Kingdom) and then digitized off-line with a sampling frequency of 10 kHz. An analog–digital interface (Power1401, Cambridge Electronic Design, United Kingdom) and Spike 2 software (Cambridge Electronic Design, United Kingdom) were used to digitize the signals and trigger the stimulation.

2.2. Data analysis

The recorded and digitized LFP signals were initially low-pass filtered with a FIR filter with a half cut-off frequency at 190 Hz and down-sampled to 250 Hz using Spike 2 software. The data were then transferred for further analysis to Matlab software environment (release R2009b, The MathWorks, Inc., USA). For the analyses presented herein – which were mainly aimed at researching the oscillations' dynamics shared by all rats – we took one signal for each rat, recorded from an electrode located in infragranular cortical layers.

The spectral power content of the spontaneous (control) LFP signals was determined by calculating and averaging squared magnitudes of Fourier transforms for successive epochs (1024 samples each, Hamming windowed) of the signal from the control phase. The 5–12 Hz band, referred to ubiquitously in this paper, was chosen for analyses as it contained the most prominent power peaks across all rats and encompassed literature data.

To quantify the instantaneous amplitude of the 5–12 Hz activity, and to identify individual oscillatory episodes present in the signals, we band-pass filtered the signals with an FIR filter with half cut-off frequencies at 5 and 12 Hz. The magnitude of the Hilbert transform of the filtered signal was calculated to obtain its envelope providing us with data of the amplitude of the signals 5–12 Hz component. To reduce jitter, the resulting 5–12 Hz amplitude waveform was smoothed with a moving average filter of a 400 ms kernel. We set a threshold of one third of the range of values registered by the 5–12 Hz amplitude waveform as giving optimal discrimination for all rats between the visible 5–12 Hz oscillatory episodes and the remaining signal. The periods in the signal where the envelope exceeded the threshold were automatically marked as the 5–12 Hz oscillatory episodes.