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Short communication

Stimulus-induced gamma activity in the electrocorticogram of freely moving rats: The neuronal signature of novelty detection

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ABSTRACT

To investigate the cortical activity pattern associated with the exploration and identification of a novel object we recorded the intracranial electrocorticogram (ECoG) in the barrel cortex of freely moving adult rats using wireless technology. We report here that the exploration and detection of a novel object correlate with a transient increase of synchronized oscillatory activity in the 40–47 Hz frequency band. This specific cortical activity pattern occurs 200–300 ms after the first sensory contact with the novel stimulus and decreases in power in the subsequent recording sessions with the same object. During the first explorative session the increase in 40–47 Hz is associated with a simultaneous decrease in the 30–37 Hz band, which increased to a stable level already after one session. Our results indicate that synchronized gamma activities in primary sensory cortex may represent the neuronal signature for the detection of a novel object.

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The gamma rhythm (30–80 Hz) is selectively enhanced in different brain areas during perception tasks [5,18,22–24] and involved in neuronal processes such as attention, learning and memory [5,9,10]. It has been further proposed that synchronized neuronal firing in the gamma frequency range may be crucial to produce a coherent object representation [5,19] and gamma as well as beta oscillatory activity accompanies the presentation of a novel auditory stimulus in the human electroencephalogram (EEG) [7]. Furthermore, it has been shown in human auditory target detection tasks that novel sounds elicited a scalp recorded potential that peaked around 200–300 ms after the presentation of the stimulus, the so-called "novelty P3" [17]. This novelty-related activity is largest over the central and frontal areas and has been observed in many different species [25,26]. In addition, in a visual detection task a relationship between the firing rate of prefrontal cortical neurons and the presentation of a novel object has been documented in primates [16]. These observations indicate that distinct oscillatory activity patterns correlate with the detection and perception of a novel object. It has been recently demonstrated in freely moving mice that beta2 oscillations (23–30 Hz) recorded in the hippocampus are transiently and selectively increased during the exploration of a novel environment [4]. These novelty-evoked oscillations only occurred when the mice were actively moving through the novel environment. Although functional studies on neocortical activity

patterns in similar behavioural conditions are still missing, structural data indicate that the cerebral cortex may be also specifically activated during exploratory behaviour in a novel environment. In the somatosensory cortex of freely exploring rodents immunocytochemical studies revealed a prominent and highly specific increase in the expression of inducible transcription factors, such as c-fos, after a single exploration in a novel environment [14,20]. However, so far no experimental study, neither in humans nor in animals, addressed the question which large-scale neuronal network activity in the cerebral cortex may be correlated with the exploration and detection of a novel object. To address this question, we used our telemetric recording system [13], which allows electrocorticogram (ECoG) recordings from unrestrained freely moving rats under video control during an explorative task. Wireless technology has been shown to minimize the stress artefacts unavoidable in conventional in vivo electrophysiological recordings [11]. Therefore telemetric recordings represent a more appropriate technique to record neuronal activity during natural explorative behaviour.

All experiments were conducted in accordance with the national and European (86/609/EEC) laws for the use of animals in research and were approved by the local ethical committee (Landesuntersuchungsamt Koblenz, 23 177-07/G07-1-001). Five Male Wistar rats weighing 300–400 g were used for this study. The animals were housed individually in standard plastic cages (42 cm × 26 cm × 20 cm) under a 12 h light-dark cycle (lights on at 7 a.m.). The room temperature was maintained at $21 \pm 2\,^{\circ}$C and relative humidity at $50 \pm 5\%$. Standard rodent food and tap water were available ad libitum. The animals were handled for 2 days

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by the experimenter who would perform the recordings. After this period the animals were placed two times 10 min/day (respectively between 09:00–11:00 and 14:00–16:00) for 4 days in an open field (60 cm × 70 cm) with one "training object" (a metal cube, Supplementary Fig. 1a) always positioned at the same location near the wall. All behavioural experiments were performed under infrared light emitting diodes in an isolated room and everything was cleaned between trials with 0.1% acetic acid.

The surgical method and the impact of the implanted system on the behaviour have been described by us in detail previously [13]. Briefly, the animals were deeply anaesthetised with chloralhydrate, a transmitter was placed in the abdominal cavity and wires were slipped between muscles and skin up to the head. The wires were soldered to three stainless steel screws (0.5 mm diameter). The recording electrode was placed in contact with the dura above the barrel cortex (L = Bregma + 5.5 mm, AP = Bregma – 2.3 mm) [15], the reference and the ground electrodes were placed in contact with the dura above the cerebellum (L = Bregma + 2 mm, AP = Bregma – 11.5 mm and L = Bregma – 1.5 mm, AP = Bregma + 11.5 mm, respectively). This assembling was fixed with grip cement (Dentsply Caulk International, Milford, USA). Both incision sites were closed using 4–0 Resolon (Resorba, Nürnberg, Germany). Surgery lasted a maximum of 3 h from induction of anaesthesia.

After surgery, a recovery period of 5 days was given to the animals before starting the first recording session, corresponding to the time needed to gain the pre-surgical weight. ECoG signal (recorded continuously at a sampling rate of 1000 Hz) and video data (25 frames/s, 720 × 576 pixels) were collected simultaneously and stored on a personal computer via CED and Spike2 software (Cambridge Electronic Design, Cambridge, England). In order to define the whisker-object contacts a CCD camera with progressive-scan sensor (JAI M10SX-C, Stemmer Imaging, Germany) was used with a fixed shutter-speed (1/250 s) to record 25 high-resolution pictures per second. The rats were placed for 2 days in the open field two times for 10 min/day with the "training object" at the same place as before (first session always between 09:00 and 11:00 and second session between 14:00 and 16:00) (Supplementary Fig. 1a and b). In the following 2 days the object was substituted by a "new object" before a new object exchange. The objects were chosen to be as different as possible to each other and to the objects the animals were exposed in the standard animal facility. Each rat was recorded 120 min in total corresponding to 40 min per object.

A video analysis was then performed to define 1 s exploration trials starting every time the animal moved toward the object and touched the object with the vibrissae contralateral to the cortical recording site. The eight successive recording sessions with the two new objects (four per object) were analysed for each animal, whereas the four recording sessions with the "training object", were not considered for this study (Supplementary Fig. 1b). These 8 sessions were separated into 2 groups: the first session for each of the 2 new objects were averaged, pooled together in the "novel condition" (n = 2 sessions per animal) and the 3 following sessions for each of the 2 objects in the "familiar condition" (n = 6 sessions per animal). Trials with ECoG artefacts were rejected. Gamma oscillations were then analysed on 5.6 ± 0.4 contacts per 10 min session (from a total average of 12.8 ± 0.7 contacts for all sessions and animals). The data were imported to MatLab (MatLab 7, The MathWorks Inc., Natick, USA) and further data analysis has been described in detail previously [12,18]. Briefly, the signal was band-pass filtered (20–80 Hz) using a Butterworth 3rd order filter and a Hilbert transform was applied on each trial followed by a pseudo-Wigner-Ville transform (time–frequency toolbox, http://tftb.nongnu.org). The resulting time–frequency maps were normalized (using maximum and minimum peaks for each map) and averaged over trials (n = 63 and 156 trials for the novel and
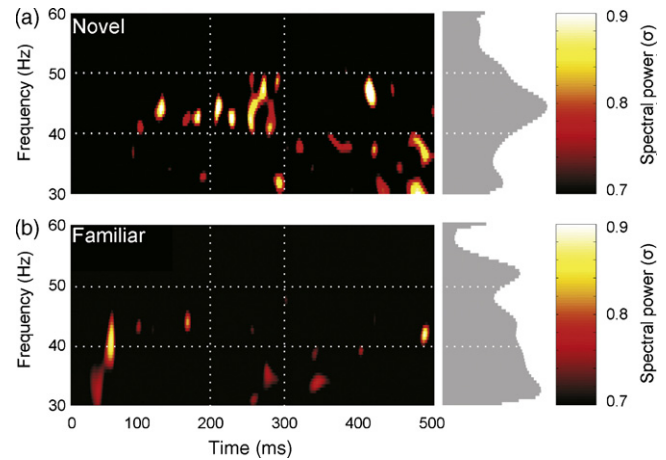
Fig. 1. Grand average time–frequency maps of the ECoG recorded above the barrel cortex of five adult rats between 30 and 60 Hz. Pseudo-Wigner-Ville transforms were averaged over all trials and subjects (n = 5) for the (a) novel (n = 63 trials) and (b) familiar (n = 156 trials) conditions. Significant differences in the ECoG gamma band activity between novel and familiar conditions could only be detected in the frequency ranges 40–47 and 30–37 Hz between 200 and 300 ms after the whiskers' first contact with the object. The relative power of each frequency between 200 and 300 ms after the first whisker contact is illustrated on the right part of each map (grey traces).

familiar conditions, respectively) and animals (n = 5). Further statistical analyses were performed in 5 Hz frequency bands that were extracted from these maps with an overlap of 2.5 Hz. P values were calculated with parametric unpaired samples t-tests (Systat Version 10, Systat Software, Erkrath, Germany) after testing the Gaussian distribution of the time–frequency relative power samples. After analysing all frequencies in the complete gamma band, only the bands showing significant differences between novel and familiar conditions were taken into account and further analysed session by session. P values were then calculated with a Dunnett's multiple comparison tests after testing the Gaussian distribution of the samples and calculating a one-way ANOVA.

The total time spent on the objects during the first and second session as well as the number of object explorations, defined as a movement toward the object followed by whiskers contact, were calculated as a measurement of novelty detection [6] and level of exploratory activity, respectively. P values were calculated with paired t-test after testing the Gaussian distribution of the samples. Values throughout this report are given as mean ± S.E.M.

It has been previously documented that rats spend significantly more time in exploring a new object as compared to a familiar one [21]. Therefore we measured the total time spent by the animals exploring the new object during the first two experimental sessions. The exploration time spent during the first session amounted to 51.6 ± 11.2 s (n = 10 sessions) and was significantly (P = 0.0314) longer as compared to the second session recorded 5 h later (31.1 ± 4.4 s). This result could not be explained by a reduction of the overall activity during the second session, since the animals presented a similar exploratory activity during the first (11.7 ± 1 exploration occurrences) and the second (12.6 ± 1.2 exploration occurrences) session. These behavioural data indicate that the animals perceived the object as novel only during the first session.

The averaged time–frequency maps revealed prominent differences in the ECoG activity during exploration of a novel versus a familiar object in the first 500 ms (see Fig. 1). We could distinguish one distinct activation period 200–300 ms after the initial contact of the whiskers with the novel object. The relative power of each frequency in the ECoG during this time window displayed two major differences between explorations of a novel versus a