Brain Research Bulletin 79 (2009) 6–14

Contents lists available at ScienceDirect

Brain Research Bulletin

journal homepage: www.elsevier.com/locate/brainresbull

Research report

A putatively novel form of spontaneous coordination in neural activity

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ARTICLE INFO

Article history:
Received 19 March 2008
Received in revised form
22 December 2008
Accepted 23 December 2008
Available online 22 January 2009

Keywords:
Local field potentials
Oscillations
Complexity
Synchrony
Resting state networks

ABSTRACT

We simultaneously recorded local field potentials from three sites along the olfactory-entorhinal axis in rats lightly anesthetized with isoflurane, as part of another experiment. While analyzing the initial data from that experiment with spectrograms, we discovered a potentially novel form of correlated neural activity, with near-simultaneous occurrence across the three widely separated brain sites. After validating their existence further, we named these events Synchronous Frequency Bursts (SFBs). Here we report our initial investigations into their properties and their potential functional significance. In Experiment 1, we found that SFBs have highly regular properties, consisting of brief (~250 ms), high amplitude bursts of LFP energy spanning frequency ranges from the delta band (1–4 Hz) to at least the low gamma band (30–50 Hz). SFBs occurred almost simultaneously across recording sites, usually with onsets <25 ms apart, and there was no clear pattern of temporal leading or lagging among the sites. While the SFBs had fairly typical, exponentially decaying power spectral density plots, their coherence structure was unusual, with high peaks in several narrow frequency ranges and little coherence in other bands. In Experiment 2, we found that SFBs occurred far more often under light anesthesia than deeper anesthetic states, and were especially prevalent as the animals regained consciousness. Finally, in Experiment 3 we showed that SFBs occur simultaneously at a significant rate across brain sites from putatively different functional subsystems—olfactory versus motor pathways. We suggest that SFBs do not carry information per se, but rather, play a role in coordinating activity in different frequency bands, potentially brain-wide, as animals progress from sleep or anesthesia toward full consciousness.

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1. Introduction

A major challenge in theoretical and systems neuroscience is elucidating the mechanisms by which long-distance coordination across different brain circuits is maintained, such that higher coherence and information transfer can rapidly occur when needed [7,45]. Coordinated activity, such as coherence in the beta- or low gamma-range (14–28 and 30–50 Hz, respectively) among local field potentials from task-related brain sites, is typically achieved within tens of milliseconds. This occurs in spite of the fact that coherence must be established across centimeters of brain space [7,9,17]. It is now known that coherence coupling is important for attention, object recognition, sensorimotor processing, and many other aspects of cognition and behavior (e.g. [14,15,13,26,27,37,42]).

Current theories of neuronal dynamics have begun to emphasize intrinsic neural activity as a crucial component of the nervous system's computational machinery (e.g. [33,35]). A variety of forms of intrinsic, non-stimulus driven activity has now been seen from small networks of neurons to extremely large networks, including what are now known as resting state networks [33,11]. How this emergent organization of neural activity arises, however, is a subject of much debate. Further complicating these issues is the fact that many of these studies are performed in the awake state, when input-driven phenomena may nonetheless be taking place. The alert state has been characterized as highly "complex" because there is considerable local-circuit as well as more global coordination [40].

To study how intrinsic brain processing emerges, we made use of the complexity concept and recorded local field potentials simultaneously from three sites within the rat olfactory-entorhinal axis – the anterior piriform, posterior piriform, and entorhinal cortices – as rats emerged from the formally analyzed, low complexity state of deep isoflurane anesthesia [46,47] to the highly complex waking state. We used recording sites within those areas that had been previously studied by Mouly, Gervais, and colleagues, who had examined multiple olfactory and entorhinal sites during anesthesia and awake behavior in rats [36,8]. We hypothesized that brain activity within that circuit would emerge via multiple metastable, intermediate stages as the rat progressed from deep anesthesia to full consciousness.

But as we analyzed spectrograms of our LFP recordings, we noticed a striking phenomenon: a potentially novel, synchronous

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0361-9230/$ – see front matter. Published by Elsevier Inc.
doi:10.1016/j.brainresbull.2008.12.017

event that was especially evident under light isoflurane anesthesia and during the transition to the waking state. We have named these events Synchronous Frequency Bursts (SFBs). These high-energy displays of momentary synchrony occurred spontaneously, lasted approximately 250 ms, appeared to be subthreshold, and occurred across widely separated olfactory and entorhinal sites with minimal temporal lag. In this report we detail our initial investigations into the properties and possible significance of this phenomenon. In Experiment 1, we first measured SFBs under light anesthesia to confirm their basic properties. We next made a systematic comparison among SFBs time series, spectrogram representations, and representations with continuous wavelet decompositions, to understand more thoroughly the time/frequency/amplitude structure of SFBs. Also toward that purpose, we analyzed their coherence structure across brain sites. In Experiment 2, we studied SFBs' occurrence as a function of level of consciousness, from deep anesthesia to waking. Perhaps most importantly, in Experiment 3 we examined whether SFBs' nearly simultaneous occurrence across brain sites was restricted to functional subsystems, or took place across likely different functional processing systems. The results showed that SFBs have a regular set of properties, a distinctive coherence structure, and a strong dependence on level of consciousness. Moreover, they appear to span functional processing subsystems, though they occur synchronously across sites within a functional subsystem more often. We discuss our findings' implications for the possible mechanisms generating SFBs and their potential functional significance.

2. Materials and methods

2.1. Subjects

We used a total of 11 female Long-Evans rats, 7 for Experiments 1 and 2 and four for Experiment 3. For all animals prior to surgery, and for the chronically implanted animals after surgery, conventional, paired housing was used, with a 12 h–12 h, reversed light-dark cycle and ad libitum feeding. Four rats in Experiments 1 and 2 were implanted with electrode bundles in their anterior and posterior cortices, and with vertical, silicon probes in their entorhinal cortices. Two more animals used in the first two experiments were chronically implanted with electrode bundles in the above three areas to explore the transition from anesthesia to waking. For Experiment 3, to study SFB occurrence across putatively different functional processing networks, two rats were implanted in their anterior piriform cortex and contralateral primary motor cortex, and the third was implanted in its anterior piriform cortex and contralateral thalamic ventro-posterior lateral nucleus. All procedures were approved by the Animal Care and Use Committee at the University of Florida.

2.2. Electrode implantations for acute and chronic preparations

For animals in Experiments 1 and 2, anesthesia was induced with isoflurane gas (5.0% isoflurane dissolved in 95.0% medical oxygen) for approximately 5 min and then modulated downward as the preparations continued, usually to ∼1.5%. Breathing rate, reflexes and mucus membrane color were checked ≤ every 10 min. Using a stereotaxic apparatus (Kopf Instruments, Tujunga, CA) for guidance, craniotomies were made over the anterior piriform (aPIR), posterior piriform (pPIR) and entorhinal (Ent C) cortices at the following locations relative to bregma: aPIR: +2.2 mm a-p, 4.0 mm lateral, and ∼6.2 mm ventral; pPIR: −2.3 mm a-p, 5.1 mm lateral and ∼8.3 mm ventral; Ent C: −6.3 mm a-p, 2.8 mm lateral, and ∼7.8 mm ventral, approaching the target at 22°. For the aPIR and pPIR (and the Ent C of chronically implanted animals), we implanted 8-wire, stainless steel and Teflon-coated electrode bundles from NB Labs (Denison, TX). The wires' impedances ranged from ∼150 to 330 kΩ. For acute Ent C recordings we used 16-contact silicon probes from Neurolynx (Bozeman, MT). Craniotomies for ground wires were drilled within 2 mm of each implant site.

With the dura mater remaining intact at other craniotomy sites before implantation, the electrode for each implantation was lowered at approximately 100 μM/min until it reached the target location. We recorded spikes and local field potentials upon descent in order to assess physiological hallmarks of correct implantation. For the two piriform cortices, there was a zone of relative silence just dorsal to the desired implant location, followed by an abrupt increase in spike activity, with larger waveforms visible in layers 2/3, the targeted layers. For the entorhinal cortex, we targeted layers 2 and 3 with the two most ventral recording contacts on the silicon probe (and likewise for the electrode tips, in the two chronically implanted rats). After passing through the highly active layers 5 and 6, we stopped approximately 300–400 μM later at the expected locations of layers 2 and 3.

For two of the animals in Experiment 3, we followed similar procedures except that we implanted an 8-wire bundle (same characteristics as above, NB Labs) into the aPIR, and a 1 × 8 array (also the same characteristics as above, NB Labs) into the contralateral M1 (0 mm a-p, 4.0 mm lateral, and ∼1.5–1.6 mm ventral). For the remaining animal in this group, we implanted 8-wire bundles into the aPIR and the contralateral VPL (−2.26 a-p, 3.0 m-l, and ∼6.2 mm ventral).

There were further procedures for the two chronically implanted rats used for the last part of Experiment 2 (studying SFB occurrence as the animals awakened from anesthesia), which we have described in detail elsewhere [25]. Briefly, in addition to making craniotomies for the electrode implants and ground wire insertions, we also inserted 5–6 metal screws (Small Parts, Inc., Miramar, Florida). Each implanted area's ground wire was wrapped around an adjacent screw and then lowered into its craniotomy. The screws also provided additional stability for the implants and headcap after the surgery. After lowering a bundle to its target range, we covered the remaining exposed brain surface with Gelfoam (Pfizer, Groton, CT) and locally stabilized the area with dental cement. After all implantations were finished, we sealed the area around the electrode connectors with dental cement, spread triple antibiotic ointment throughout the wound, and used sterile wound clips to close the wound. Upon waking the animals were administered buprenorphine every 8 h for 4 days for pain control, and their wounds were cleaned and dressed daily for 7 days.

2.3. Recording procedures for data collection

The main physiological signal used in our study was local field potentials (LFPs). All recordings were made using neural data acquisition hardware and software from Plexon, Inc. (Dallas, TX). The LFPs were low-pass-filtered at 100 Hz and sampled at 1 kHz. For three acutely olfactory-entorhinal animals, LFPs were recorded for 60 s under light (0.25%) isoflurane anesthesia, with moderate to strong hindpaw reflexes but no spontaneous movement. Their isoflurane levels were then increased successively to .5%, 1.0%, 2.0%, 3.0%, 4.0% and 5.0%, with recordings made at each level for 60 s. Five acutely recorded olfactory-entorhinal animals, and all olfactory-motor animals in Experiment 3, were recorded under light (∼.2%) isoflurane levels for 6 min, while ensuring that the animals did not spontaneously move or evince signs of pain. They were then switched to 4.0% isoflurane and their anesthetic state was allowed to deepen until there were no forepaw, hindpaw, earlobe or corneal reflexes and their respiration rate fell below 40 breaths/min. Once that state was achieved, they underwent 6 more minutes of recording. Following these recordings they were deeply anesthetized at 5.0% isoflurane and decapitated.

Once the chronics had recovered from surgery, we used them for the last part of Experiment 2. We anesthetized them for 3 min with 5.0% isoflurane, and then allowed them to emerge from anesthesia as we recorded LFPs from the aPIR, pPIR and Ent C. We recorded from the time we took the animal out of the stereotaxic apparatus, which occurred when the animal began blinking and making other small movements, until it was fully awake 30–45 min later.

2.4. Data analyses: an overall view

All analyses were performed using NeuroExplorer (Plexon Inc., Dallas, TX), Excel (Microsoft, Tacoma, WA), Matlab (The Mathworks, Natick, MA), AutoSignal (SYSTAT, Chicago, IL) and Statistica (StatSoft, Tulsa, OK). Our first task was to search for cross-brain area coordination events, not knowing in which timescale or frequency band they would occur, if at all. We thus performed spectrogram analyses (combining fast-Fourier transforms with moving window averaging) with window sizes ranging from 3 to 5 s, and sliding the window every 3–50 ms. We were readily able to see SFBs in all these conditions and settled on coding the data with two window shift sizes, 3 and 25 ms. As will be explained later, the 3 ms shift distance was used for some analyses, and 25 ms was used for the others.

After finding and coding numerous SFBs using spectrogram analyses, we performed descriptive statistics on the coded events, including measuring their mean duration at the frequency of 20 Hz, and the mean differences in their temporal onset across the aPIR, pPIR and Ent C. We next systematically examined the SFBs' time series, spectrograms, and continuous wavelet decompositions, to better understand which characteristics of the time series generate SFB-like objects, and to verify the main properties of the spectrograms using an alternative time-frequency visualization method. Finally, we examined the spectral and coherence structure across SFBs. These steps comprised Experiment 1.

In Experiment 2, we examined the relationship between SFB occurrence and level of consciousness in three ways. The resulting data were analyzed with ANOVAs and, where appropriate, post hoc, Bonferroni-corrected *t*-tests. Last, in Experiment 3, we measured SFB occurrence in the aPIR and M1 or VPL, tabulating the number of SFBs that occurred in each area and how many of them overlapped in time. We compared these numbers to similar numbers taken across the aPIR and pPIR, and the aPIR and Ent C, to explore whether SFBs occur at a significant rate across functional subsystems.

2.5. Coding of SFBs with spectrograms

We performed spectrogram analyses [39] with two parameter settings: first, with a 500 ms window shifted every 3 ms, and then with a shift distance of 25 ms. In both cases we took 126 frequency samples, and at the larger sliding distance