

Chapter 2

Methods

2.1 Experimental Procedures

Chronic recordings were performed in the Langone Medical Center of the New York University and the Department of Physiology of the School of Medicine of the University of Szeged. All experiments were performed in accordance with European Union guidelines (2003/65/CE) and the National Institutes of Health Guidelines for the Care and Use of Animals for Experimental Procedures. The experimental protocols were approved by the Animal Care and Use Committee of New York University Medical Center and the Ethical Committee for Animal Research at the Albert Szent-György Medical and Pharmaceutical Center of the University of Szeged respectively. Animals were anesthetized with isoflurane anesthesia and one or several craniotomies were performed with stereotaxical guidance. One or more silicon probes were mounted in custom-made micro-drives to allow their precise vertical movement after implantation. The probes were inserted over the target region and the micro-drives attached to the skull with dental cement. The craniotomies were sealed with sterile wax. Two stainless steel screws were drilled over the cerebellum and serve as ground and reference for the recordings. Several additional screws were drilled into the skull and covered with dental cement to strengthen the implant. Finally, a copper mesh was attached to the skull with dental cement and connected to the ground screw to act as a Faraday cage and prevent the recording from the environmental electric noise (Fig. 2.1a. For more details, see Vandecasteele et al. [45]). After recovery, the probe is moved gradually in 70–150 μm steps until the desired target is reached. The operated animals were housed in individual cages.

To record neuronal activity during sleep or waking behaviors the probes were connected to a pre-amplifier headstage attached to a long cable pending from the room ceiling that allow full movement to the animal (Fig. 2.1b). The rats' positions during behavioral sessions were estimated using video tracking of two LEDs fixed

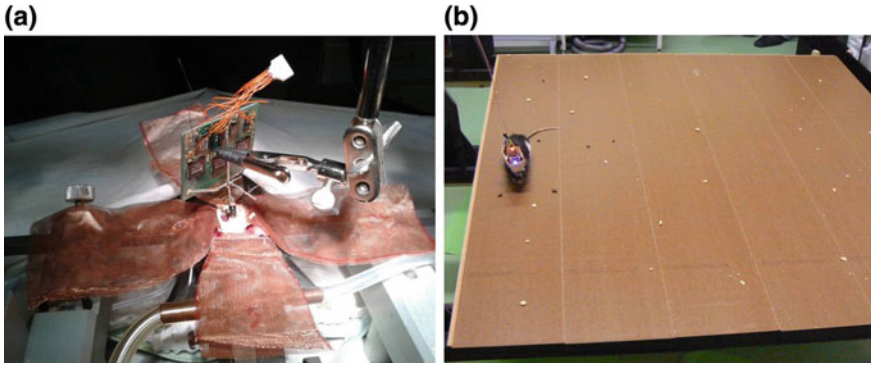


Fig. 2.1 **a** Implantation of a 256 channels silicon probe (NeuroNeexus) in the hippocampus of a Long Evans rats. Observe the multiplexed pre-amplifier PCB and the microdrive where the probe is mounted. **b** An animal being recorded during exploration for food reward (cookies) in an open field. Two LEDs mounted in the headstage are used for position tracking

to the headstage. The wide-band signal was low-pass filtered and down sampled to 1250 Hz to generate the LFP and was high-pass filtered (>0.8 ; 20 kHz) for spike detection.

Following the termination of the experiments, the animals were deeply anesthetized, and transcardially perfused first with 0.9 % saline solution followed by 4 % formaldehyde solution. The brains were sectioned by a Vibratome (Leica) at 70 μm sections, parallel with the plane of the implanted silicon probes.

For some of the analysis presented here another dataset was also included. It comprised recordings from the medial entorhinal cortex and hippocampus performed with high-density 32 or 64 electrode (Buzsáki-probes, Neuronexus) while the rats performed different maze tasks or sleep in their home cages. This dataset is publically available at <http://crcns.org> (hc-3 dataset, [32]).

The experimental data analyzed for the present thesis were not entirely recorded by me but by other researchers from the Buzsáki laboratory (NYU): AntalBerényi, Sean Montgomery, Kenji Mizuseki and John Long.

2.2 LFP Source Decomposition

All the LFP pre-processing and analysis were conducted with custom-made functions or publically available toolboxes in MATLAB (The MathWorks, Inc.).

To address the inverse problem of LFP, that is, to separate the different sources that contribute to the mixed signal, we employed a combination of independent component analysis (ICA) and current source density (CSD) analysis.

2.2.1 Independent Component Analysis

ICA is a blind source-separation technique [4, 16, 26] that can isolate spatially segregated stable patterns of activity in a mixed signal recorded with an array of sensors. Applied to linear profiles of LFPs it can separate physiologically meaningful sources that can be attributed to known anatomical pathways. ICA is able to find the original sources that are statistically independent in a linear mixture. This can be achieved by different ways, thus many different ICA algorithms have been proposed that, although numerically different, are equivalent from a theoretical point of view [15, 25]. We employed the logistic infomax ICA algorithm [4] as implemented in the EEGLAB toolbox (RUNICA; [18] and the fast kernel density ICA algorithm provided by Chen (KDICA, <http://cm.bell-labs.com/who/aychen/ica-code.html>; [13]). Both algorithms give very similar results.

The application of ICA to a blind source separation problem can be formulated as follows:

Given a linear mixture of n sources $x_1, \dots, x_n$ that are independent from each other (that is, observing the dynamics of one of them does not give any information about the dynamics of the others), the problem is to separate the source signals given only the mixture

$$x_j(t) = a_{j1}s_1(t) + a_{j2}s_2(t) + \dots + a_{jn}s_n(t) \quad (2.1)$$

For the following explanation we will eliminate the temporal dimension and denote by x the column vector whose elements are the mixture signals $x_1, \dots, x_n$ and by s the column vector whose elements $s_1, \dots, s_n$ are the independent sources:

$$x = A s \quad (2.2)$$

A is the mixing matrix with elements a_{ij} . The above equation can also be formulated as

$$x_j = \sum_{i=1}^n a_{ji}s_i \quad (2.3)$$

The above equation is called the statistical ICA model. It is a generative model because it describes how the observed mixed signal is generated by an iterative process of mixing of the independent components s_i . The components cannot be directly observed, they are latent variables. Also the mixing matrix A is a priori unknown. We only observe the mixed signal x and have to estimate both A and s . After that we can, just by means of an inverse transform, obtain the independent components:

$$s = W x \quad (2.4)$$

where W is the inverse matrix of A .

A common first step in the ICA is to sphere or whiten the mixed signals; that is, to remove any existing correlations. If $\mathbf{C} = E\{\mathbf{x}\mathbf{x}'\}$ is the correlation matrix of the mixed signals, the sphering can be accomplished by the linear transformation $\mathbf{V} = \mathbf{C}^{-1/2}$

$$E\{\mathbf{y}\mathbf{y}'\} = E\{\mathbf{V}\mathbf{x}\mathbf{x}'\mathbf{V}'\} = \mathbf{C}^{\frac{1}{2}} \cdot \mathbf{C} \cdot \mathbf{C}^{-\frac{1}{2}} = \mathbf{I} \quad (2.5)$$

After sphering the independent components can be estimated by an orthogonal transformation of the uncorrelated signals $\mathbf{y}$. This is achieved by a rotation of the joint density $p(\mathbf{y})$. The appropriated rotation is sought by maximizing the non-Gaussianity of the marginal densities of $p(\mathbf{y})$. This relies on the assumption made by ICA (as stated by the central limit theorem) that any linear mixture of independent random variables has necessarily a more Gaussian distribution than the original variables. Most ICA algorithms perform this rotation of the joint density of the signals in an iterative way until it converges. That is achieved when the joint density becomes a product of the marginal densities:

$$p(s) = p(s_1) \cdot p(s_2) \cdots p(s_n) \quad (2.6)$$

The ICA model makes several assumptions that must be fulfilled for the data in order to reach an accurate result [7, 26]: (1) Sources must be stationary. In the context of LFPs, the sources are mainly synaptic transmembrane currents in fixed locations as determined by anatomy [9]. (2) The sources should not have Gaussian-distributed activation strengths, which is the case for brain dynamics [8]. (3) The mixture of the sources must be linear and instantaneous, which can be assumed for electric fields elicited by ionic currents in the extracellular space [34, 36]. (4) The number of sources must be equal or less than the number of sensors, which makes high-density electrode recordings particularly suitable for application of ICA. (5) It is assumed that the observed variable (in this case the LFP) is a mixture of scalar, one dimensional sources. It implies that if the same oscillatory source is observed with a phase delay by different electrodes, then ICA will decompose it into two ICs with a 90 degree phase delay. The temporal activation of the original source would correspond to a linear combination of the time series of those two ICs. Thus, it is important to stress that, though spatially distinct sources which are *perfectly* coherent cannot be properly separated, ICA does not find independent components (ICs) with true temporal independence, and temporal correlations and coherence measures may still be applied to analyze the temporal relationships between the resulting ICs [4, 15, 23, 26, 39].

Our case is that of multiple simultaneous samplings of the mixed signal (the raw LFP recorded by every electrode, Fig. 2.2a). The time series of the LFP recorded by each electrode are the rows of the data matrix $\mathbf{D}$. ICA finds the square matrix $\mathbf{W}$ (with dimensions equal to the rows of $\mathbf{D}$) such that $\mathbf{W}\mathbf{D} = \mathbf{C}$. $\mathbf{W}$ is the unmixing matrix because it separates the mixture of signals that is $\mathbf{D}$ into its independent sources. $\mathbf{C}$ has the same dimensionality as $\mathbf{D}$, with each of its rows being the time series of an independent component (Fig. 2.2c, lower panel). Each independent

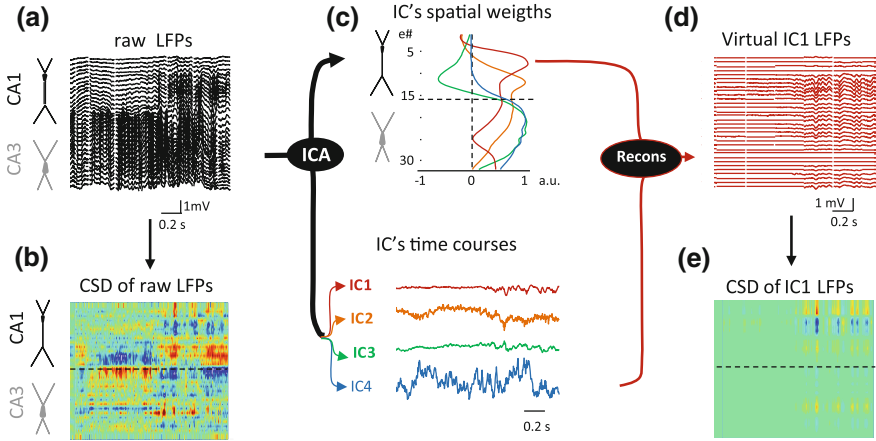


Fig. 2.2 **a** Raw LFPs along the CA1 and CA3 hippocampal subfields (*black and gray traces*, respectively). **b** CSD of the LFPs renders a complex mixture of currents as expected for multiple synaptic inputs. **c** ICA of LFPs provides four main ICs, each defined by the curve of spatial weights (*top panel*) and a time course (*bottom traces*). **d** Reconstructed (virtual) LFPs for IC4. **e** CSD of the virtual LFPs provides precise spatiotemporal maps of inward/outward currents for unique spatially coherent synaptic input. Modified from [20]

component is obtained by multiplying each sampled signal by each row of $\mathbf{W}$ (the so called unmixing functions or ICA filters). This process can be also view as solving the inverse problem of the LFP; that is estimate the sources giving only the potentials distribution.

The inverse of the mixing matrix $\mathbf{W}$ that transforms the LFP data into the ICs gives the channel weight of each component that is captured for each sensor. When plotted according to the anatomical location of the electrodes, this corresponds to the spatial voltage loadings of each IC (Fig. 2.2c, upper panel). We ranked the components by the amount of variance they explain in the original data (*relative power*). Once ICs have been extracted from the raw LFP traces, they can be analyzed as if they were active independently from activities at other locations. We reconstructed the virtual LFP produced by a single IC by multiplying the IC time course by its correspondent voltage loading (Fig. 2.2d). For each component a , that is achieved by

$$\mathbf{W}_a^{-1} \mathbf{C}_a = \mathbf{D}_a \quad (2.7)$$

That is also known as solving the forward problem, or reconstructing the potentials distribution given the current sources.

Before application of the ICA algorithm, we performed several pre-processing steps. For hippocampal LFPs, there are only a small number of physiologically meaningful ICs with significant amplitude and identifiable spatial loadings [5, 21–23, 39]. Before applying ICA, we therefore employed a principal component analysis

(PCA) reduction maintaining 98.0 % of the original LFP variance. This process aids in the convergence of ICA to stable components, and results in a smaller number of ICs. We also whitened the data before applying ICA to reduce the computational complexity of the analysis while maintaining its statistical consistency [12, 25].

Due to the parallel anatomical arrangement of the principal cells and the stratification of afferent axon terminals, the hippocampus is especially well suited for ICA decompositions of its LFPs. However, only those synaptic inputs with enough postsynaptic current, synchrony, and spatial clustering are suitable for ICA separation; thus, very weak or sparse currents are not easily discernible.

2.2.2 Current Source Density Analysis

The traditional approach to solve the inverse problem of the LFPs is performing current source density (CSD) analysis. CSD analysis [24, 31] determines the magnitude and location of the net transmembrane currents generated by neuronal elements within a small volume of tissue.

In a macroscopic level the transmembrane current density I_m per unit length is related with the extracellular current density $\mathbf{J}$ through the divergence:

$$\nabla \cdot \mathbf{J} = I_m \quad (2.8)$$

If we assume a quasistatic description of the electric field in the extracellular medium (neglecting capacitive and inductive effects), Ohm law can be applied,

$$\mathbf{J} = \sigma \mathbf{E} \quad (2.9)$$

where σ is in general the conductivity tensor.

The relation between the electric field $\mathbf{E}$ and the extracellular potential Φ

$$\mathbf{E} = -\nabla \Phi \quad (2.10)$$

allows us to establish a linear dependency between the extracellular current density $\mathbf{J}$ and the gradient of the electric potential in the medium

$$\mathbf{J} = -\sigma \nabla \Phi \quad (2.11)$$

Substituting this expression of $\mathbf{J}$ into the current conservation equation, we get the Poisson equation, which establishes the relation between the electric potential and the volumetric current density

$$\nabla \cdot (\sigma \nabla \Phi) = -I_m \quad (2.12)$$

In the simplest approach, we employed a 1D approximation by calculating the second spatial derivative of the LFP profiles (Fig. 2.2b), and calculated the CSD according to a central differences formula,

$$CSD(z_i) = -\sigma \frac{\Phi(z_i + \delta) - 2\Phi(z_i) + \Phi(z_i - \delta)}{\delta^2} \quad (2.13)$$

where δ is the distance between electrodes.

This approach assumes isotropy and negligible net contributions in the XY-plane perpendicular to the cellular axis. That is suitable for laminated structures with parallel arrangement of principal cells, as is the case of CA1, if the recording electrodes are placed parallel to the main cell axis (z) and an homogeneous population of cells is synchronously active (as for example during evoked potentials). In this traditional approach to CSD estimation it is also assumed homogeneous resistivity.

However, the above assumptions are not fulfilled in most real cases. When the cells in a small volume of tissue are active only in a given time there can be a significant current spreading in the x and y directions. This may result in an underestimation of true sink and sources as well as in the occurrence of spurious ones. To address those issues we employed the recently developed inverse CSD (iCSD) method [35]. This method consists of first define a forward model to describe the potentials that are produced by localized current sources and then invert this model by means of a numerical matrix inversion to allow direct calculation of localized discrete sources from the measured potentials distribution. The solution of the forward model is given by the following equation

$$\Phi(r_e) = \frac{1}{4\pi\sigma} \iiint_V \frac{CSD(r)}{|r - r_e|} d^3r \quad (2.14)$$

In most LFP studies the application of CSD analysis does not take into account tissue inhomogeneity and anisotropy effects, assuming a negligible contribution of these effects [9, 19]. In the present work we also adopted this approach; however we took advantage of the iCSD method to explicitly incorporate a priori knowledge about the geometry of the sources, such as the volume of activated tissue and the relative position of the sources to the recording electrodes.

In addition to the raw LFP, we performed CSD analysis of the reconstructed virtual LFP produced by a single IC, which renders a map of the current distribution of a single anatomical input or LFP source [20, 21, 39].

2.3 Time-Frequency Analysis of LFPs

To characterize the LFP signals in the spectral domain we employ a multi-taper implementation of the fast Fourier transform ([30]; Chronux toolbox, <http://www.chronux.org>). Continuous data were segmented in 1–5 s epochs for all spectral

analysis. Spectral power was estimated with a Hanning window and averaged across all data epochs.

In the multi-taper power spectrum method employed here a set of independent estimates of the power spectrum are computed, by multiplying the signal by orthogonal tapers (windows) which are constructed to minimize the spectral leakage due to the finite length of the data set. The tapers are the discrete set of eigenfunctions that solve the variational problem of minimizing leakage outside of a predefined frequency band. Once the tapers $w_k(t)$ are computed for a chosen frequency bandwidth, the total power spectrum P_X can be estimated by averaging the individual spectra given by each tapered version of the time series $x(t)$; the k th eigenspectrum X_k is the discrete Fourier Transform of $x(t)w_k(t)$

$$P_x(f) = \frac{\sum_{k=1}^K \mu_k |X_k(f)|^2}{\sum_{k=1}^K \mu_k} \quad (2.15)$$

where μ_k are the eigenvalues of w_k .

This procedure yields a better and more stable estimate of a signal power spectrum than single taper methods.

Two basic measures of pairwise synchronization were employed: cross-correlation and coherence. The cross-correlation function is a measure of the linear covariance between two signals x and y and can be estimated as follows,

$$CC_{xy} = \frac{\sum_{k=1}^n (x_k - \bar{x})(y_k - \bar{y})}{\sqrt{\sum_{k=1}^n (x_k - \bar{x})^2} \cdot \sqrt{\sum_{k=1}^n (y_k - \bar{y})^2}} \quad (2.16)$$

where $\bar{x}$ and $\bar{y}$ are the means of the respective signals.

This method has the advantages of being a straightforward method that yields a normalized value giving a gross indication of the degree of similarity of two signals (i.e. LFPs recorded at separate locations). However, for a frequency resolved estimate of the linear covariance of two signals we need to employ the coherence.

$$coh_{xy}(f) = \sqrt{\frac{|\langle C_{xy}(f) \rangle|^2}{|\langle C_{xx}(f) \rangle| \cdot |\langle C_{yy}(f) \rangle|}} \quad (2.17)$$

where $C_{xx}(f)$ is the cross-spectral density for the frequency f between x and y , and $\langle \dots \rangle$ indicates averaging over segments.

To assess spectral events at a high resolution in time and frequency, the complex wavelet transform (CWT) of the LFP was calculated using complex Morlet wavelets [42]. The CWT gives amplitude and phase measures for each wavelet scale at all time points in the data, obtained by convolving the real and imaginary parts (which are phase shifted by 90°) of the wavelets with the data vectors.

A wavelet is a scalable function with zero mean, well localized in time.

$$\int_{-\infty}^{\infty} \psi(t) dt = 0 \quad (2.18)$$

A family of wavelets can be constructed from a “mother” function $\Psi(t)$, which is confined to a finite interval, translated with a factor u and expanded with a scale parameter s ,

$$\psi_{u,s}(t) = \frac{1}{\sqrt{s}} \cdot \psi\left(\frac{t-u}{s}\right) \quad (2.19)$$

Then the wavelet analysis of a signal x can be performed by

$$\langle x(t), \psi_{u,s}(t) \rangle = \int x(t) \psi_{u,s}(t) dt \quad (2.20)$$

In our case, we employed the Morlet complex waveform,

$$\psi(t) = \left(e^{i\omega_0 t} - e^{-\omega_0^2 t^2 / 2} \right) \cdot e^{-t^2 / 2\sigma_t^2} \quad (2.21)$$

where ω_0 is the center frequency of the wavelet and σ_t a bandwidth parameter determining its rate of decay. The width or number of cycles of the wavelet is given by $\omega_0 \sigma_t^2$.

If this complex wavelet is convolved with the signal x we get the CWT of that signal,

$$W(t) = (\psi \circ x)(t) = \int \psi(t') \times (t - t') dt' = A^W(t) e^{i\phi_W(t)} \quad (2.22)$$

where $\phi_W(t)$ is the phase for each time sample. The Morlet mother waveform has Gaussian modulation in both time and frequency, thus offering optimal resolution in both domains.

The simpler Hilbert transform was also employed in some cases (as when only the theta oscillations were considered) to extract the phase or amplitude of LFP

signals. The phase $\phi_w(t)$ and amplitude $A_x(t)$ are given respectively by the argument and modulus of the complex analytical signal:

$$\xi(t) = x(t) + ix_H(t) = A_x(t)e^{i\phi_x(t)} \quad (2.23)$$

where $x_H(t)$ is the Hilbert transform of $x(t)$, defined as:

$$x_H(t) = \frac{1}{\pi} P.V. \int_{-\infty}^{\infty} \frac{x(t')}{t - t'} dt' \quad (2.24)$$

with $P.V.$ denoting the Cauchy principal value.

Both wavelet and Hilbert transforms give very similar results in all cases.

The phase-amplitude cross-frequency coupling (CFC) during theta oscillations for a given LFP recording was assessed using the modulation index (MI) introduced by Tort et al. [44]. We took the phase of the LFP recorded at the CA1 pyramidal cell layer and the amplitude of either LFP recorded in different layers or the time course of different LFP-generators, in all cases the procedure was exactly the same. The raw LFP signal (Fig. 2.3a) was band-pass filtered in the low frequency band (Fig. 2.3b) and the phase of the analytic signal given by the Hilbert transform or CWT was calculated (Fig. 2.3c). Amplitude of the filtered signal in the broad gamma band (30–300 Hz, Fig. 2.3d) was also obtained from the CWT. The MI was calculated by measuring the divergence of the observed amplitude distribution from the uniform distribution (Fig. 2.3e). Comodulogram phase-amplitude plots were constructed representing in pseudocolor scale the MI values of multiple phase-amplitude frequency pairs (Fig. 2.3f). The statistical significance of the MI values (P-value) was assessed by a surrogate analysis ($n = 1000$ surrogates) with random shifts between the phase and amplitude time series [11].

2.4 Single Unit Analysis

Neuronal spikes were detected from the digitally high-pass filtered LFP (0.8–5 kHz) by a threshold crossing-based algorithm (*Spikedetekt2*; <https://github.com/klusta-team/spikedetekt2>). Detected spikes were automatically sorted using the masked EM algorithm for Gaussians mixtures implemented in KlustaKwik ([27]; <https://github.com/klusta-team/klustakwik/>), followed by manual adjustment of the clusters using the KlustaViewa software ([37]; <https://github.com/klusta-team/klustaviewa/>; Fig. 2.4) to get well-isolated single units. Multiunit or noise clusters were discarded for the analysis. Putative pyramidal cells and interneurons were separated on the basis of their autocorrelograms and waveforms characteristics [17, 33, 40], assisted by monosynaptic latency excitatory and inhibitory interactions between simultaneously recorded, well-isolated units [2, 33]. Kenji Mizuseki at the Buzsáki laboratory performed most of the unit clustering and classification.

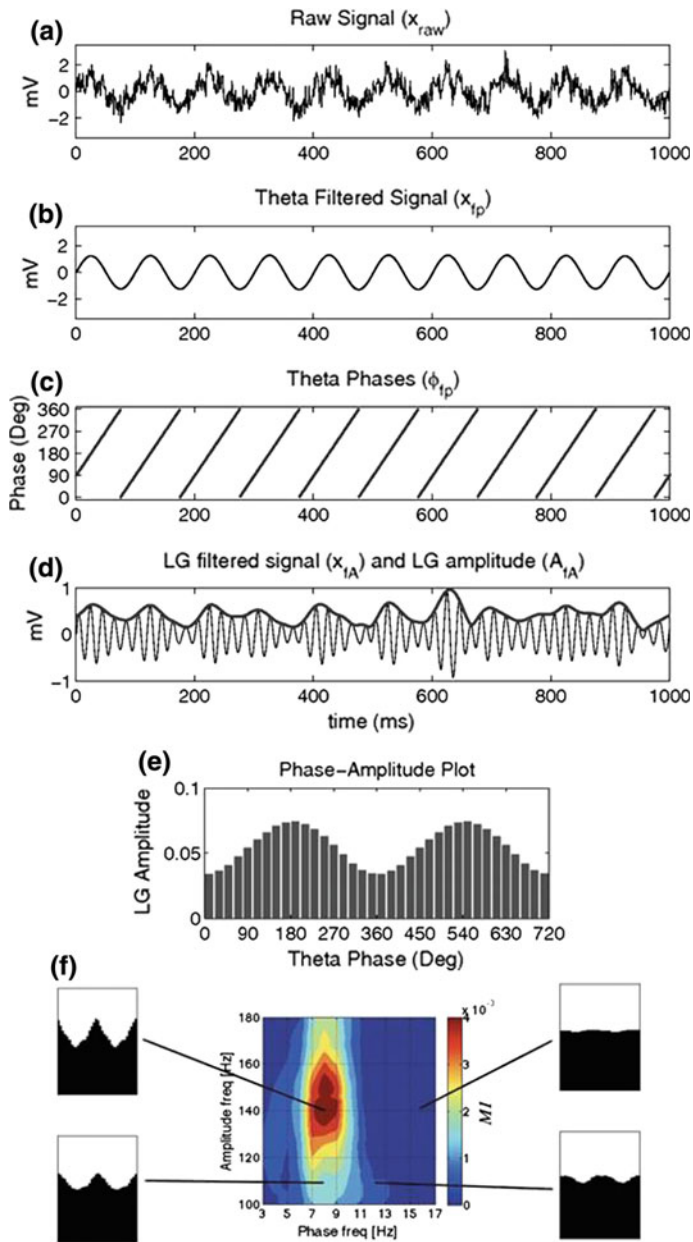


Fig. 2.3 a Example of raw LFP signal. After filtering in the theta band (b), the phase is calculated using the Hilbert transform. c The same or other signal is filtered in the frequency band of interest and its amplitude (d) is obtained from the CWT. The mean amplitude distribution over theta phase is then calculated (e). The phase-amplitude MI is obtained by measuring the divergence of the observed amplitude distribution from the uniform distribution. A phase-amplitude comodulogram plot is constructed representing in pseudocolor scale the obtained MI values for multiple phase-amplitude pairs (in this case the phase was not only calculated for the theta filtered signal but for a range of frequencies). Modified from [43, 44]

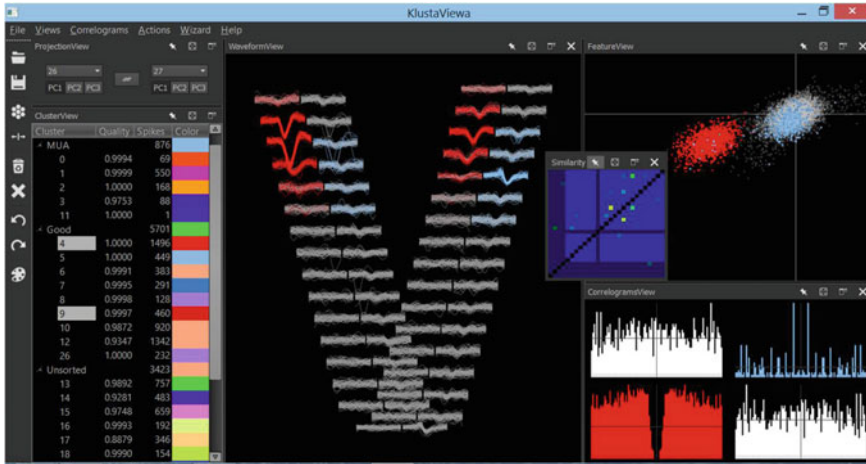


Fig. 2.4 Screen capture from KlustaView, an open-source software for manual clustering of neuronal spikes. In the *central panel*, two single units with different anatomical location are displayed. Observe the different features provided for assisting the process: auto and cross-correlograms. PCA projections, similarity matrix. From [37]

The phase-locking of spikes to the LFP was measured for individual units using the wavelet or Hilbert phase at the time of each spike. Modulation indices were calculated using the mean resultant length of the phases, and significance was estimated applying the Rayleigh test for non-uniformity using the circular statistics toolbox provided by Berens [6]. Erik Schomburg at the Buzsáki laboratory implemented and performed unit-LFP analyses.

2.5 Modeling of LFPs with Finite Elements Method

Multiple physical problems can be mathematically formulated as a partial derivatives differential equation. As a general rule, those differential equations are very hard to solve. Only in those cases in which it is possible to make simplifications on the dimensionality and geometry of the problem an analytical solution is available. However, in most of the cases analytical methods are not feasible or are inexact (as can be the case of a tridimensional electromagnetic problem in a complex geometry). For solving this kind of problems numerical models are necessary and useful. Those methods discretize the differential equation into a linear system of equations, solving it in an iterative way. One of those methods is the analysis by means of finite elements (FEM).

The basis of FEM is to divide the geometry in which a differential equation of a scalar or vector field needs to be solved (i.e. the electric potential) in small elements, typically tetrahedral. In each element field equations determined by a

variational principle and local sources are solved. Boundary conditions are applied and thus it is possible to obtain the algebraic system from which a solution of the problem is found. FEM allows explicit models of the geometry and electrical properties of the brain to be generated and to solve potential and current distributions in such a complex structure over time. In our case, the physical problem that needs to be solved with FEM can be described as follows. The initial condition is a certain distribution of volumetric current sources in a tridimensional complex structure (i.e. the rat hippocampus). Each of the sources is simultaneously activated with different time series. As a result of the uneven spatiotemporal varying summation of currents in the volume, an electric potential distribution arises. Thus, our goal is to calculate this potential distribution for each spatial point at anytime.

As such, we modeled the rat hippocampus establishing the geometry of current sources and their temporal activation, and fixed the boundary conditions. Two different models were built, one of the whole dorsal hippocampus in its planar section, and another of just the dentate gyrus region. The structure was enclosed in a larger volume simulating the extracellular space. This outer compartment was sufficiently large so as not to distort the field lines in the central region of interest. We tested different surrounding volumes (up to four times longer in each direction) while maintaining boundary conditions. The chosen volume rendered LFPs that were at least 90 % of the maximum amplitude obtained with the largest volume (>95 % in most tested points). For the sake of simplicity, the tissue was considered to be purely resistive, isotropic and homogeneous [29] but see [3]. Conductivity was set as 0.3 S/m and relative permittivity as 10^6 . FEM current sources were defined as tridimensional volumes, allowing the electrical currents produced by multiple synchronously activated neurons to be compiled in a few block-like current generators that jointly obey the principle of charge conservation. This is an important advantage, which makes it suitable to reproduce LFPs in a volume generated by activated sections of layered structures made up of neurons arranged in parallel as is the case of the hippocampus or cortex. Accordingly, the size and geometry of the blocks of current represent the physical extension of the synchronously activated dendritic domains, i.e., the portion of the population of target cells that elicits postsynaptic transmembrane currents upon coherent activation of a group of afferent axons (Fig. 2.5a).

We found that eighth of these blocks with 100 μm thickness in the case of CA1 and four 60 μm thickness block for each blade of the DG were sufficient to reproduce the main laminar features of hippocampal LFPs [23]. In the case of CA1, one for the basal dendrites, one for the pyramidal cell body layer and six for the apical dendrites. For the DG, one block represented the granular cell body layer and three the dendrites. These blocks were bent to replicate the curved geometry of the hippocampus and divided into approximately 200 μm sections that could be independently activated to analyze the effects of the spatial coherence and synchrony of the inputs. For simplicity, we used non-overlapping blocks of current, representing inputs with imaginary topological projection of varying synaptic territories.

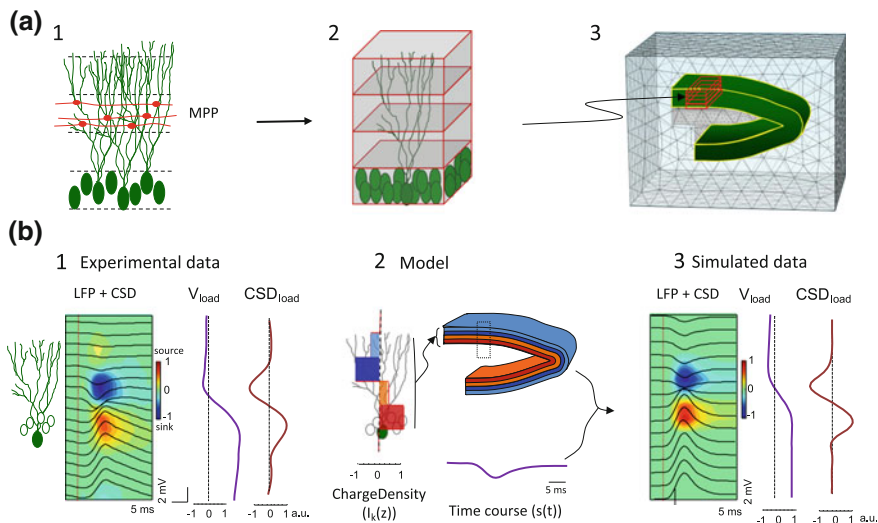


Fig. 2.5 **a** The gross cytoarchitecture of the granule cell (GC) population (I) was assembled as four stacked rectangular blocks each representing a subcellular domain. **2** The blocks were given appropriate curvature and dimensions to reproduce a stereotyped U-shaped geometry of the dorsal dentate gyrus (DG). **3** A tetrahedron adaptive recording mesh simulating the conductive extracellular medium was built in and around the cellular component and was large enough not to distort electrical fields. **b** The block sources were activated using real time activations. In this example, we used the excitatory medial perforant pathway (MPP) that makes synaptic contact in the middle third of the dendritic tree (see **a1**). Total charge was balanced across all blocks at every instant. Charge was distributed throughout all four compartments according to weights obtained in the spatial map of CSD analysis for the electrical activation of this pathway. V_{load} and CSD_{load} represent the spatial weights of voltage and CSD along the GC main axis. The excitatory sink (in blue) is surrounded by a strong passive source (warm colors) in cell soma and a weaker source in distal dendrites. **2** At all times, the blocks received proportional charge density with predefined polarity and identical time course. **3** Example of FEM simulated data. Adapted from [23]

In the present models we deliberately excluded any possible contribution of the extracellular currents from other cellular types apart from pyramidal and granular cells (e.g. interneurons or glia). Most types of interneurons and astrocytes have multipolar dendritic trees [1] and hence, a ‘closed-field’ extracellular configuration of the electric field is established by their synaptic activation (Lorente de Nó, 1947). Consequently, their currents do not (or they only poorly) spread beyond their physical limits [10, 19, 28]. In consequence, these cells do not significantly add their currents in the extracellular space and they only negligibly contribute to the LFP (even if they are strongly synchronized).

We applied Dirichlet boundary conditions by setting the field to the ground value on the external surface of the enclosing volume and imposing charge conservation inside the total volume. A tetrahedral adaptive grid of the highest resolution (smallest size, 0.05 μm) was used to ensure the correct resolution of field equations in the curved compartments (Fig. 2.5a3).

The FEM approach is widely used in magnetoencephalography and scalp electroencephalography [14, 38, 41], although its application to the study of LFPs is almost inexistent. We used a commercial FEM software tool, COMSOL Multiphysics® (www.comsol.com).

Time-dependent analyses were performed using the AC/DC module of COMSOL for all nodes in the extracellular and cellular volume. The spatial and temporal dynamics of LFP and CSD distributions were evaluated after activation of individual subcellular domains by different inputs (the rationale of the performed simulations is illustrated in Fig. 2.5b; see [23]). The temporal activation of a particular synaptic afference, such as the MPP excitatory input, was taken as the input signal, $s(t)$. For simplicity, we represent here an MPP-evoked fEPSP. The axons of this pathway establish synaptic contact with GCs in the middle third of their dendritic tree (Fig. 2.5b1, green axons). The spatial distribution of the CSD corresponding to this activation (Fig. 2.5b1, contour map) was compartmentalized into four spatial blocks that jointly configured the GC population, such that they roughly reproduced the same spatial profile (Fig. 2.5b2). The sum of charge densities was set to zero, as imposed by current conservation law. The electric fields and potentials elicited by these currents were calculated for the entire tissue volume by FEM.

Linear profiles of simulated LFPs comparable to those recorded in vivo were built using several linear tracks along the vertical z -axis, which contained up to 40 registration points spaced at 50 μm intervals and that were placed in the middle of the structure to produce the most homogenous field contribution. LFP profiles were constructed from the instantaneous voltage signal recorded at each simulated recording point.

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