Independent component analysis of neural populations from multielectrode field potential measurements

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Abstract

Independent component analysis (ICA) is proposed for analysis of neural population activity from multichannel electrophysiological field potential measurements. The proposed analysis method provides information on spatial extents of active neural populations, locations of the populations with respect to each other, population evolution, including merging and splitting of populations in time, and on time lag differences between the populations. In some cases, results of the proposed analysis may also be interpreted as independent information flows carried by neurons and neural populations. In this paper, a detailed description of the analysis method is given. The proposed analysis is demonstrated with an illustrative simulation, and with an exemplary analysis of an in vivo multichannel recording from rat hippocampus. The proposed method can be applied in analysis of any recordings of neural networks in which contributions from a number of neural populations or information flows are simultaneously recorded via a number of measurement points, as well in vivo as in vitro.

Keywords: independent component analysis, ICA, cognition, brain wave, brain frequency, frequency analysis, neural population, neural activity

1. Introduction

In this paper, we address the question of observing neuron populations based on extracellular multielectrode field potential measurements (MFPM). By their nature, such measurements do not explicitly yield single population data, but rather a mixture of field potentials (FPs) from several populations is measured. It is assumed that such measurements carry FP information from a number of neural populations, in which neurons are working in unison for a common task in a highly unified manner. To observe individual populations from FP measurements, an ICA based method is proposed. While feedbacks between the neural populations may destroy independence of the populations, it may still be possible to observe independent information flows within the populations. It is assumed that such independent information flows may occur regardless of possible feedbacks, while to make conclusions on independent populations one must carefully consider the underlying neuroanatomy. Conclusions on the neural populations may be made with higher confidence if the brain structure of interest has definitive anatomically disjoint populations, or if the analysis is performed on a time window, which is shorter than the minimum feedback time between the populations. We call an observable neuronal population, or an independent information flow, a functional population (FUPO). For our analysis, a FUPO does not have to be confined in a space not shared by other FUPOs, but for illustrative purposes populations are drawn spatially disjoint. The essence of the proposed analysis is to allow interpretation of the independent components (ICs), given by ICA of an MFPM, as active FUPOs. Thereafter, the FUPOs can be analyzed, e.g., with regard to their brain wave frequency content and their evolution in time. The terms “IC” and “FUPO” are used interchangeably, depending on the context. The proposed method is exhaustively described, and examples are designed to illustrate both correct analysis and caveats.

ICA (Hyvärinen, 1999a; Hyvärinen and Oja, 2000; Hyvärinen et. al., 2001; Stone, 2002) has become a de facto tool in several disciplines. Also in brain research, ICA has been applied in a multitude of cases, including electric current source localization (Zhukov et. al., 2000), electroencephalography (EEG) and magnetoencephalography analysis (Vigário et. al., 2000), with which Jung et. al. (2001) also considered functional magnetic resonance imaging. More closely related to our work, ICA has been used in epileptiform discharge detection from EEG (Kobayashi et. al., 1999), spike train analysis of population interactions (Laubach et. al., 1999), and individual neuron action potential detection (Brown et. al., 2001). Also relevant is neuronal ensemble activity analysis using principal component analysis (PCA) (Chapin and Nicolelis, 1999). To the best of our knowledge, ICA has not been previously applied in direct analysis of brain MFPMs. Our analysis yields the active individual FUPOs in the surroundings of the recording multielectrode in the brain structure of interest, allowing one to deduce information on spatial extents of active neural populations, locations of the populations with respect to each other, population evolution, including merging and splitting of populations in time, and on time lag differences between populations. Any of the common FP analysis methods can be applied to analyze the individual FUPOs, including time–frequency and wavelet analyses (Akay, 1998), and current source density analyses (Freeman and Nicholson, 1975; Kaibara and Leung, 1993) methods. Furthermore, ICA FP analysis can also be augmented with intracellular measurements.

In our experiments, the animals were required to be well anesthetized during the whole measurement. Thus, the analysis presented in this paper is not concerned with changes in the state of the animal, e.g. sleep vs. wake, or the level of activity. It could be enlightening to perform the same analysis on multielectrode measurements from behaving animals in order to observe possible changes in FUPOs, and to see if any FUPOs remain constant regardless of the animal state. Naturally, any brain structure, from which multielectrode measurements can be obtained, can be subjected to ICA FUPO analysis. For example, the cerebral cortex would be a lucrative structure for ICA of FUPO FPs with measurement points (MPs) arranged in a plane or in a three dimensional mesh. Ultimately, it would be of utmost interest to devise an automated ICA FUPOs analysis for large-scale neural ensemble recordings (Buzsáki, 2004) based on the proposed analysis method.

1.1. FUPOs and multichannel FP recordings

It is assumed, that a population of neurons is operating in unison, thus producing a FUPO FP observable via a number of measurement channels, and that there exist one or more neuronal populations at unknown distances giving rise to independent measurable FUPO FPs. While tetrodes measure the immediate surroundings of the measurement site, allowing for individual neuron activities to be identified, here, an FP measurement is assumed to pick up FPs from more distant groups of neurons. For
the analysis proposed in this paper, extracellular FP measurements are assumed to be made with a multielectrode amongst these FUPOs, as illustrated in Fig. 1. Fig. 1A illustrates the overall scenario of a multielectrode in the vicinity of several FUPOs, and Figs. 1B–E illustrate the different realization possibilities. The scenario in Fig. 1D with each MP recording a sum of a few FUPO FPs is probably the most likely one to occur with our measurements from the hippocampus when the brain is functioning normally, while the scenario in Fig. 1E may reflect epileptiform hypersynchronization. For an extensive description of epileptic activity, see McCormick and Contreras (2001).

Naturally, each channel of the electrode in Fig. 1A, records a different sum of FPs produced by the electrical activity in the populations. ICA assumes that each channel records a linear combination of the FPs produced by the populations; an assumption, which is valid at least if the electrical properties of the tissue remain constant during each measurement period, and the measured FP depends linearly of the actual FP at the producing neural population. Also, it is assumed that the measurement system is accurate enough, and the FUPOs are sufficiently strong and their FPs spatially extended, in order to be observed at a few MPs a few 100 µm apart.

![Fig. 1.](image-url)

**1.2. Principals of ICA**

In this Section, basic concepts of ICA are outlined. The presentation is based on Hyvärinen (1999b), Hyvärinen and Oja (2000), and Hyvärinen et. al. (2001). A recommendable account on the history of ICA can be found in Hyvärinen et. al. (2001), where it is noted that the concept of ICA first arose in the context of neurophysiology. In addition to the papers on physiological applications already mentioned, other starting points include papers from along the history of the development of related algorithms (Amari et. al., 1996; Bell and Sejnowski, 1995; Cardoso and Souloumiac, 1993; De Lauthauwer et. al., 2001).

The strength of ICA lies in its ability to extract $I$ independent components (ICs) $x_i$, $i = 1, 2, \ldots, I$, from $J$ measured signals $y_j$, $j = 1, 2, \ldots, J$, $I \leq J$. A measured signal is assumed to be a linear combination of the components,

$$y_j = a_{i,j}x_i + \cdots + a_{J,j}x_J, \quad j = 1, 2, \ldots, J,$$  \hspace{1cm} (1)

where $a_{i,j}$ are coefficients of the linear combinations. In this paper, the measured signals are simultaneously recorded channels of an MFPM. While the signal model for ICA (1) does not explicitly take noise into account, the ICs can be assumed noisy without loss of generality. ICA will treat noise as any other signal component, producing ICs, which contain varying amounts of noise and payload signal. In this paper, $y_j$ is an $N$ samples long FP measurement vector measured via the $j$th channel,

$$y_j = [y_j(0) \quad y_j(1) \cdots \quad y_j(N-1)]^T, \quad j = 1, 2, \ldots, J,$$  \hspace{1cm} (2)

where $y_j(n)$, $n = 0, 1, \ldots, N-1$, is an individual measured voltage at time $n$. Superscript “T” denotes transpose. Similarly,

$$x_i = [x_i(0) \quad x_i(1) \cdots \quad x_i(N-1)]^T, \quad i = 1, 2, \ldots, J.$$  \hspace{1cm} (3)

In order to find $K$ ICs, at least $K$ simultaneously recorded channels must be available. In this paper, $J = 2, 3$ simultaneously recorded FP channels are used in calculating $K = J$ ICs. Also, the case that there exist $K < J$ ICs is demonstrated. Note that in the following chapters, the subscript of $y$ denotes the number of the multielectrode channel, for example, with $J = 3$, the three measured signals taken to ICA are denoted by $y_6$, $y_8$, and $y_{15}$.
In matrix form, a measurement matrix is given by

\[ Y = XA, \]  

(4)

where measurements vectors are the columns of \( Y = [y_1, y_2, \ldots, y_J] \), \( X \) consists of ICs in columns, \( X = [x_1, x_2, \ldots, x_K] \), and if \( K = J \), \( A \) is a \( K \)-by-\( K \) mixing matrix with elements \( a_{ij}, i = 1, 2, \ldots, K \). E.g., with signals measured via multielectrode channels 6, 8, and 15, \( Y \) consists of three columns, \( Y = [y_6, y_8, y_{15}] \), \( X \) consists of three ICs in columns, \( X = [x_1, x_2, x_3] \), and \( A \) is a 3-by-3 mixing matrix. The simulated example in Section 2, shows a case where \( K < J \) with four simulated measured signals, three true ICs, and thus a 3-by-4 mixing matrix \( A \).

The task of ICA is now to find such \( A \) that columns of \( X \) are independent of each other, and

\[ X = YA^{-1}. \]  

(5)

In this paper, a fast iterative algorithm, FastICA (Hyvärinen, 1999b; Hyvärinen et. al. 2001), is used to estimate the ICs. Denoting an estimate with the hat, estimates of the ICs are given by

\[ \hat{X} = Y\hat{A}^{-1}, \]  

(6)

and thereafter estimates of the measurements can be reconstructed as

\[ \hat{Y} = \hat{X}A, \]  

(7)

if necessary. ICA calculation using two signals \( y_n \) and \( y_m \) is denoted by \( ICA(y_n, y_m) \), \( n, m \in \{1, 2, \ldots, J\} \), while ICA may be calculated with any number of measurement signals.

Assumptions of ICA, in addition to the signal model (1), are that the components are independent, and the independent components have nongaussian distributions. The first assumption is the essence of ICA. Since the ICs are actually the maximally nongaussian components, ICA with Gaussian components is impossible. Uncorrelated Gaussian signals are also independent. The second assumption is addressed later when describing the ICA algorithm. The ambiguities of ICA are that energies of the found ICs cannot be determined, and order in which the ICs appear in \( \hat{X} \) (6) is undetermined. The former is a direct consequence of that (4) can be written with an arbitrary constant \( c_k, k = 1, 2, \ldots, K \), as

\[ Y = X'A', \]  

(8)

where \( X' = \begin{bmatrix} 1 & \frac{1}{c_1} & \frac{1}{c_2} & \cdots & \frac{1}{c_K} \end{bmatrix} \), \( A' = \begin{bmatrix} c_1a_{1,1} & c_1a_{1,2} & \cdots & c_1a_{1,K} \\ c_2a_{2,1} & c_2a_{2,2} & \cdots & c_2a_{2,K} \\ \vdots & \vdots & \ddots & \vdots \\ c_Ka_{K,1} & c_Ka_{K,2} & \cdots & c_Ka_{K,K} \end{bmatrix} \). The latter ambiguity results from the fact that in (4), for any order of the columns of \( Y \), the order of columns of \( X \) can be any, given the corresponding \( A \). Nothing can be done about the first ambiguity, and it has to be kept in mind when observing the ICs. Unfortunately, the first ambiguity has no effect on our analysis. In this paper, a remedy to the second ambiguity is proposed. Assuming that a few concurrent measurements exist so that not all the ICs are present in all the measured signals, it is possible to calculate ICAs on all combinations of the measured signals, and thus it may be possible to deduce which measurements carry which ICs. Also, calculating ICAs separately on several sets of signals with sufficient overlap in time between the sets, it may be possible to find strong correlations between subsequent ICs, thus allowing one to observe the ICs over a prolonged period of time. For our measurements, both turn out to be possible, and are elaborated upon later in the paper.

The widely applied principal component analysis (PCA) (Hyvärinen et. al. 2001) assumes the same signal model (1), and finds uncorrelated component signals. In essence, PCA maximizes the variance of the components, i.e., captures first the largest trends in the data, when estimating one principal component at a time. While correlation may be regarded as a linear relationship between the signals, independence is a much stronger requirement, excluding also non-linear relationships between the components. Laubach et. al. (1999) state that PCA is able to find correlations between large NPs, while ICA can be used to separate more limited components of NPs based on higher-order correlations. On the other hand, Brown et. al. (2001) note that for optical measurements of spiking neurons, PCA was not able to separate spike trains, whereas ICA was successful. In this paper, we address ICs of FPs. While separate FUPOs may carry correlated FPs, we address the independent processes present, so as either to separate independent FUPOs, should such exist, or independent information flows in the possibly dependent FUPOs. To interpret the ICs either as FUPOs or independent information flows, one must carefully consider the anatomy of the target brain structure with regard to the measurement setup.
1.3. FastICA

This Section gives a short introduction to the employed FastICA algorithm based on its creators’ works (Hyvärinen, 1999b; Hyvärinen et. al., 2001). The FastICA package for Matlab (MathWorks Inc., Natick, MA, USA) is available at the FastICA WWW site (FastICA Package for Matlab, 2003). At heart, FastICA is an algorithm to estimate the ICs, i.e., $\hat{X}$ in (6), by iteratively maximizing non gaussianity of the components. While non gaussianity of the components cannot be ensured beforehand, FastICA strives to iteratively maximize negentropy (Hyvärinen, 1999b; Hyvärinen and Oja, 2000). Thus, successful application of FastICA also proves that non gaussian components exist. There also exists a kurtosis based version of FastICA (Hyvärinen and Oja, 2000). Advantages of FastICA include fast convergence (at least quadratic, sometimes cubic, contrary to linear convergence of gradient descent methods), no need for the user to find an appropriate step size parameter; performance of the algorithm can be tuned with respect to data by selecting the nonlinearity present in the calculations (as described in Hyvärinen (1999b), where it is noted that the selection is not essential for successful ICA), and that ICs can be estimated one by one (as in projection pursuit), or in parallel. Even computing FastICA in a sliding window is well feasible, since on the average, it took FastICA less than 10 ms of processor time per ICA on a 2.4 GHz Pentium IV processor, when computing 125 ICAs with two 1250-point input signals for each of the plots in Fig. 9. Furthermore, FastICA is extensively documented in the literature (FastICA Package for Matlab, 2003; Hyvärinen, 1999a; Hyvärinen and Oja, 2000; Hyvärinen et. al., 2001). It is to be noted that while for most of the signals used in this paper FastICA converges fast, usually already after two to 20 iterations, it is possible to encounter input signals for which FastICA does not converge. Nonconvergence will result from excessive Gaussian noise, e.g., from noise with the amplitude comparable to that of the measured signal, but in our case this was not the case. In some cases, turning on stabilization or using a different nonlinearity in FastICA, changing the number, length, sampling rate, or band width of the input signals, or preprocessing the input signals to reduce noise, resulted in convergence. With a few measured MFPMs, we did not get ICA to converge. In such cases, one can assume that the basic assumptions of ICA are not met. To illustrate nonconvergence probability with our data, all two-input and three-input ICAs were calculated for each of the 240 one-second periods of the signals seen in Fig. 4A, using all four nonlinearities available in FastICA, i.e., the total of 3840 ICAs were calculated. The percentages of nonconvergent ICAs were a little less than 2 % using either of the nonlinear functions ‘tanh’ or ‘gauss’ (as they are called in the Matlab program of FastICA), approximately 3 % using the nonlinearity ‘pow3’, and 6 − 7 % using ‘skew’. It is to be noted that the percentages may vary greatly with the type of input signals used.

The stabilized version of FastICA involves an automated step size adjustment; with stabilization on, FastICA halves the step size for the time the algorithm is stuck between two points, or for the rest of the run, if convergence is not observed within first half of the iterations. Noise reduction may be done in FastICA by dimension reduction, i.e., by first calculating PCA, and calculating ICA on the signals, which are reconstructed from the most significant components from PCA. In order to illustrate appearance of ICs which are mostly noise, noise reduction by PCA was not applied in the examples shown in this paper.

2. ICA of simulated signals

Let us approach the proposed analysis by illustrating the basic function of ICA with a simulated example. The simulation is designed only to give the reader an illustrated idea of ICA, and thus FUPOs used in this example are not designed to simulate real FUPOs. The proposed analysis will be elaborated upon later when analyzing the real life measured multielectrode FPs, and only a few main issues are pointed out here. First, assume three FUPO FPs shown in Fig. 2A. Note that it is not ensured that the FUPOs in Fig. 2A are independent, like we cannot ensure that the MFPMs would contain FUPOs as ICs. These FUPO FPs are assumed to be mixed at the MPs of the multielectrode according to (9) to produce four simultaneously measured signals

\[
4 \times 33 \times 1000 = XAXY
\]

In Fig. 2C, ICs from four FUPOs in Fig. 2A are independent, like we cannot ensure that the MFPMs would contain FUPOs as ICs. These FUPO FPs are assumed to be mixed at the MPs of the multielectrode according to (9) to produce four simultaneously measured signals

\[
\begin{bmatrix}
X,
Y,
A
\end{bmatrix}
\]

respectively, of this example, are shown in square brackets. Time is running down in columns of X and Y in (9). In Fig. 2C, ICs from four representative two-input ICAs, from a three input ICA, and from the four-input ICA are shown. To truly analyze the FUPOs, one may consider using all available measurements from the relevant locations of brain structure of interest, and calculating all possible ICAs. For this example, the total number of ICAs would be 11, i.e., using all possible sets of two, three, and four input signals $y_j$, $j = 1, ..., 4$.

\[
\begin{bmatrix}
Y
\end{bmatrix}
= \begin{bmatrix}
X
\end{bmatrix} A = \begin{bmatrix}
0.7 & 0.6 & 0.2 & 0 \\
0.2 & 0.7 & 0.8 & 0.3 \\
0 & 0 & 0.4 & 0.9
\end{bmatrix}
\]

(9)
Fig. 2. (A) Simulated ICs, i.e., the true simulated FUPO FPs. (B) Simulated measured FPs, i.e., mixtures of the ICs in (A). (C) Selected ICAs calculated from the mixtures (B). In (C), all ICs are scaled with the maximum over all the ICs. Note that ambiguities of ICA are seen as indeterminate signs, and as undetermined order of the found ICs. In (C), ICs from each ICA calculation are shown in subplots arranged in columns, with ICA(y₁,y₂) denoting an ICA calculated with input signals y₁ and y₂, seen in (B), which in turn are calculated from the signals in (A) as given by (9), and indicated within the subplots of (B). Individual ICs are labeled with IC₁₁ through IC₆₃ within the subplots for later reference.
Comparing Fig. 2A, for example, with ICA($y_1,y_2$), and ICA($y_2,y_3,y_4$) in Fig. 2C, it is seen that when the number of ICs to be estimated matches the true number of ICs, the true ICs can be fairly well estimated, but the sign of the ICs is arbitrary. In Fig. 2C, it is seen that ICA($y_1,y_2,y_3,y_4$) yields only three ICs, which demonstrates the fact that FastICA inherently finds only the true number of ICs when excessive number of linear combinations is presented to the algorithm. In this example, even if noise is added to the original ICs before mixing them, FastICA finds only three ICs (data not shown), given that the noise level is not excessive, which may also cause ICA not to converge. In general, noise may cause additional ICs to be found, while noise may also be incorporated into the ICs carrying the payload signal. However, according to our experience, the noise present on the payload signal ICs is usually attenuated. Observing ICA($y_1,y_2$), ICA($y_2,y_3$), and ICA($y_3,y_4$), in Fig. 2C, it is seen that when there are more original components, c.f. Fig. 2A, than input signals, the found ICs display characteristics of the original components in an undetermined fashion. In order to determine the spatial position of the FUPOs relative to the MPs, it is necessary to be able to determine how well ICs from different ICAs correspond to each other. Even between the ICs of ICA($y_1,y_2$), ICA($y_2,y_3$), and ICA($y_3,y_4$), in Fig. 2, it is clearly possible to determine the pair–wise correlation coefficients, or $p$-values for similarity, and thus determine which kind of ICs coincide with which measurement channels.

3. FP Measurement and signal analysis methods

3.1. Comments on measurements for ICA analysis

The measurements presented in this paper were originally made in vivo from the rat hippocampus for epilepsy and brain wave frequency research to be submitted for publication elsewhere. Here, only the materials and methods directly relevant to this paper are presented. The hippocampus (Rolls and Treves, 1997) is a trisynaptic structure involved in learning and memory. Basically, the information arrives at the hippocampus from entorhinal cortex via perforant path through dentate gyrus (DG), and flows out to subiculum and entorhinal cortex, and the two hippocampi are connected via dorsal and ventral commissures (fimbria fornix). See Fig. 3 for the overall structure of the hippocampus along with the measurement and stimulation setup. Interpreting the results as activities of specific hippocampus FUPOs, thus deducing the operation of the hippocampus, requires exact knowledge of the placement of the multielectrode MPs with respect to the hippocampal anatomy. Electrode MP placements in particular cell layers were determined based on observed electrophysiological landmark properties (Markram, 1997). Thus, the measurement setup in this paper allows us to address independent FUPO, or neural operation modes, present in the three cellular layers shown in Fig. 3.

Since the original FP measurements were not anatomically verified by histology, horizontal placements of the MPs within hippocampal cell layers are not available. An alternative setup would be to place the MPs along a horizontal line, so as to measure adjacent FUPOs in a single cell layer. Also, the MPs could be arranged in a horizontal plane, or as a three dimensional measurement mesh. To get the most out of the proposed analysis method, measurement setup should be carefully designed in order to fully utilize the capabilities and to fulfill the requirements of ICA as perfectly as possible for the brain structure of interest.

3.2. Signal acquisition and measured FP signals

The methods used in the experiments were approved by the Provincial Government of Eastern Finland (approval number 99–61). The measurements presented in this paper are from one randomly selected rat out of a batch of 25 Kuopio Wistar rats (250–350 g) anaesthetized with 1.1–1.4 g/kg urethane. In a stereotactic instrument (Kopf series 962), the scalp was removed, and a 16–channel silicon probe (courtesy of University of Michigan Center for Neural Communication Technology) with 100 µm MP separation was inserted via a drilled bone window. Recordings were made via 14 channels along the hippocampal subfields (−3.6 mm anteroposterior (AP), −4.0 mm lateral (L) to bregma, in 30° angle towards midline) to monitor principal cell layers of DG and Cornu Ammonis region 1 (CA1), as well as CA1 Schaffer Collaterals (Sch). See Fig. 3 for a schematic illustration of the setup. Positioning of the multielectrode was observed from polarity of the FPs, firing patterns and population spike shapes (Markram et. al., 1997), and their latencies. A pair of stainless steel wires (100 µm in diameter) with 0.2–0.4 mm tip separation was placed in the fimbria fornix (−1.3 mm AP, +1.0 mm L to bregma, and −4.0 mm ventral (V) from cortical brain surface) to stimulate antidromically the commissural efferents of the contralateral CA3 region, and an additional pair of steel wires was placed −7.0 mm AP, +4.0 mm L to bregma, −3.0 mm V from cortical brain surface to stimulate the perforant pathway. The intensity of 0.2 ms electrical pulse stimulation (Master8 pulse generator and Iso–flex stimulus isolator, A.M.P.I, Jerusalem, Israel) was twice the threshold current capable of inducing population spikes in more than two consecutive trials for both stimulation sites, i.e., applied stimulation current was 300–600 µA. Two stainless steel watch screws driven to bone above the cerebellum served as indifferent and ground electrodes. The multichannel measurement signals were 500–fold amplified using Multichannel Systems amplifiers (MPA–8 and PA–32–D, Reutlingen, Germany), high–pass filtered at 0.1 Hz and low–pass filtered at 5 kHz (PA–32–D), digitized to 16–bit precision (Digidata 1320A, Axon Instruments) at 12.5 kHz sampling rate, and stored to a personal computer. All subsequent data manipulation and analysis was made in Matlab.
Fig. 3. Structure of the rat hippocampus –3.6 mm posterior to bregma, including an illustration of the recording and stimulation electrodes within the hippocampus. Open circles on the multielectrode illustrate the channels used in the ICA example, with the channel 6 in Cornu Ammonis region 1 (CA1) pyramidal cell layer, channel 8 amongst Schaffer Collaterals (Sch), and channel 15 in the hilar region of dentate gyrus (DG). Lines within the hippocampus illustrate fascicle, and dots the cellular layers. Cornu Ammonis field 3 (CA3) location is also indicated. Note that the multielectrode and the hippocampus may not be in the same scale, and that the horizontal positioning of the multielectrode may not have been exactly as shown.

For demonstrating the proposed ICA analysis method, signals measured via the multielectrode channels 6, 8, and 15, i.e., with 200 µm and 700 µm MP separations, respectively, are taken into analysis. Channel 6 was measured in CA1 pyramidal cell layer, channel 8 amongst Sch, and channel 15 in the hilar region of DG. The used measurement channels are illustrated with open circles on the multielectrode in Fig. 3, and the measured signals are seen in Fig. 4A. With regard to the measured signals seen in Fig. 4A, ipsilateral perforant path (c.f. Fig. 3) was stimulated approximately between time points 60 s and 85 s, and contralateral fimbria fornix during the period between 180 s and 205 s, with stimulation patterned in bursts so that pulses at 14 Hz occur in bursts at 2 Hz, which bursts are delivered in blocks at 0.25 Hz. ICAs were calculated for the four 1 s time periods marked with ‘x’ in Fig. 4A, which periods are seen in detail in Figs. 4B–E. The last period taken into analysis is after electrical stimulation, which was aimed at producing epileptiform activity, but before any such activity occurred. In the beginning of Section 4, the proposed analysis is demonstrated by calculating all possible two- and three-input ICAs for each of the four 1 s time periods shown in Figs. 4B–E. In Section 4.1, calculation of PSD time series from the series of ICs from ICAs in a sliding window is demonstrated using the 2 s signals seen in two parts in Figs 4B and 4C, thus providing a method to observe the time evolution of brain wave frequencies present in the FUPOs.

Fig. 4. (A) FPs measured from the rat hippocampus via channels 6, 8, and 15 of the multielectrode, denoted by $y_6$, $y_8$, $y_{16}$, respectively, in (B)–(E) and in the sequel. Channel locations within the hippocampus are illustrated in Fig. 3. Electrical stimulation is seen between time points 1 min and 1 min 25 s, and also between 3 min and 3 min 25 s. The one second periods marked with ‘x’s in (A) are shown in detail in (B)–(E). The period seen in (C) follows immediately that seen in (B). The measurement period seen in (D) is taken during electrical stimulation, and the period in (E) starts approximately 6.5 s after the last electrical stimulation has ended. (D) exhibits FPs, population spikes, and stimulation artifacts. Later in this paper, ICA is applied to each set of three simultaneously measured signals seen in (B)–(E).
Fig. 4. (Continued).

3.3. Signal conditioning

Sampling rate was lowered to 1250 Hz from the original 12.5 kHz, and the frequency band of interest was set to range from 0 Hz to 200 Hz in three steps: the signal was decimated by five, lowpass filtered using the 10th order Chebyshev type II filter with stopband ripple 60 dB down and cutoff stopband edge frequency set to 200 Hz, and finally decimated by two. The lowpass filtering was performed both forward and backward to preserve signal phase. The decimation functions also included appropriate lowpass filters (MathWorks, 2002b). This arrangement made it possible to do some experimentation on the effects of different sampling frequencies without affecting the bandwidth. Limiting the frequency band of interest below 200 Hz allows us to concentrate on FPs, and to exclude action potentials. Naturally, the analysis may be preformed similarly regardless of the frequency band of interest, given that the FastICA algorithm will converge.
3.4. Signal analysis methods

In this Section, computational methods for analyzing the measured signals and the found ICs are described. After ICA, the analysis methods applied to the ICs are power spectral density (PSD) estimation, correlation analysis, and hierarchical clustering. All the signal processing and analysis was performed on one second periods, i.e., using 1250 samples at a time, except for the IC cross-correlation p-value calculations, for which a reduced number of samples was used.

3.4.1. Power spectral density estimation

For frequency analysis, PSDs were calculated using the Welsh’s method (Hayes, 1996; MathWorks, 2002b). The unit of the PSD is power per Hz. In the Welsh’s method, 1250 sample signals were padded with zeros (Jackson, 1996) to the length of 65536, which was processed using a window of 16384 samples in order to achieve sufficient peak frequency detection, and the window was moved over the zero padded data eight samples at a time, i.e., the subsequent window overlap was 16736 samples. For the PSDs shown in Figs. 5B, 6B, 7B, and 8B, frequencies of the local maxima are listed in Tables 1, 2, 5, and 6, respectively. For illustrative purposes, and to concentrate on the most significant frequency components, only the maxima reaching over 10% of the maximum PSD level in each set of nine PSDs are listed in Tables 1, 2, 5, and 6. Here the 10% can be considered as an arbitrarily chosen background noise PSD level. Background noise PSD could be determined at the time of the original multielectrode measurements, in order to identifying the true PSD peaks. Unfortunately, background noise was originally not measured.

3.4.2. Determining similarities between ICs

To analyze the ICs, similarities between the ICs from different ICA calculations are to be determined. Here, two approaches are demonstrated: hierarchical clustering (Eichenbaum and Davis, 1998; Hand et. al. 2001; MathWorks, 2002c), and correlation analysis (Milton and Arnold, 1995; Eichenbaum and Davis, 1998; MathWorks, 2002a). Hierarchical clustering produces disjoint sets of ICs, so that the ICs assigned to one cluster are more similar to each other than to the ICs in the other clusters, in a sense determined by the selected distance metric. On the other hand, correlation analysis yields a similarity measure between all the ICs, and can be used also for cluster validation, and for IC similarity determination between ICs from different ICAs, and across cluster boundaries. Thus, the proposed approaches can be used in addressing similarities between ICs from different ICAs during the same time period, or between ICs from ICAs of FPs measured during different time periods. The latter is necessary in order to follow a FUPO in time. If ICA is calculated on separate time periods to observe FUPO time evolution, the periods should be overlapped to yield more confident identification of corresponding subsequent ICs.

In hierarchical clustering, distances between the ICs are calculated, and ICs with the smallest distances are pair-wise linked to each other. The found IC pairs form the first level clusters, which are again pair-wise linked on the next level. This procedure is continued to form a linkage tree. A cutoff point is set to discontinue linking at a desired cluster level. As the distance, we selected correlation distance (i.e., one minus the pair-wise correlation between the ICs) to better relate the clustering approach to the correlation analysis approach. Distance metric can be freely selected according to the desired similarities to be detected, the most commonly known distances being Euclidian and city block distances. By visual inspection of Fig. 5A (or Fig. 6A, Fig. 7A, or Fig. 8A), it is easy to group the ICs resulting from different ICAs according to the signal shapes, but it turns out not to be trivial to find an objective and rigorously justifiable criterion for setting the cutoff point. Similar matter is also shortly discussed by Eichenbaum and Davis (1998). Here, we let the cutoff point decrease until more than one cluster was found. For example, for the ICs in Fig. 5A, we ended up with the cutoff of 1.15, which yielded the three clusters indicated in Fig. 5. Similar clustering was applied to the ICAs seen in Figs. 6, 7, and 8. Especially with larger sets of ICs, this method may underestimate the number of clusters. In such a case, cutoff can be manually decreased to yield a clustering with more clusters, but it is to be noted that such a clustering is purely subjective, if the clustering is accepted based on visual inspection only. With our data, clustering results were quite robust against additive Gaussian noise. For example, when adding zero mean Gaussian noise to the Fig. 5A data to be clustered, for far most of the runs the clustering results remained reasonable and the cutoff between 1.00 and 1.15, up to added Gaussian noise variance of 40% of the maximum absolute value of the data. Instead of ICs, PSDs or other frequency content measures of the ICs can also be used as inputs to the clustering algorithm.

In correlation analysis, pair-wise cross-correlations between all the ICs were calculated, and the time lags of the maximum correlations were recorded. p-values for the pair-wise correlation coefficients were calculated with the Matlab function ‘corrcor’ (MathWorks, 2002c): a p-value is the probability of getting the corresponding correlation as a result of random chance, and it is calculated transforming the correlation to obtain the T statistics with N–2 degrees of freedom, where N is now the number of samples of each signal used in the calculations. The time lags, rounded to the nearest millisecond, and orders of the p-values of the maximum correlations for those IC pairs, whose correlation coefficient p-values were less than 0.0001, are given in Table 3. To produce Table 3, every sixth time point of the data was used in the calculations of the p-values, while all the data points were used in maximum correlation time lag determination, as well as in cluster analysis. Ambiguity of the IC sign presents a problem in the delay analysis; if the similar ICs are found with different signs, i.e., in opposite phases, the apparent time lag is modified by half of the signal period. To avoid this, correlations are calculated with both signs of one IC, and the shorter of the found time lags is shown in Table 3. Also, one may choose to restrict the maximum time lag to cover only the synaptic conductance times within the brain structure of interest, but this excludes also signals, which originate from further away, and arrive to the measurement site via different paths after considerably different delays.
When doing correlation analysis between ICs calculated on different time periods, the time lag is not meaningful. Therefore, for these cases, only the orders of correlation \( p \)-values are shown in Table 3.

It is to be noted that in this kind of analysis, \( p \)-values are not very descriptive since they vary greatly with the signal length. Therefore, the orders of the \( p \)-values given in Table 3 are to be considered as relative strengths of the correlations above a more or less arbitrary \( p \)-value threshold, rather than rigorous statistical significances. For this kind of analysis it would suffice to observe pair-wise correlation coefficients, but \( p \)-values are given because of neuroscience conventions.

3.4.3. Observing FUPO brain wave frequency time evolution

Time evolution of FUPOs was addressed by calculating ICA of two input signals in a one-second window, sliding the window over a desired period one sample at a time, and calculating PSDs of every 10th pairs of ICs. Thus, 128 PSDs of each of the two ICs were produced per second. Decreasing the overlap would affect the calculations in two ways, which both would make identification of corresponding subsequent ICs less reliable: Firstly, with more different subsequent input signals, the found ICs would naturally also be more different. Secondly, it would be more probable that the subsequent calculations of ICA algorithm converged to rather different solutions. Thus, if the computational cost is acceptable, like with the number and length of our input signals, it is recommendable to slide the input window one sample at a time to get the best possible results. Thereafter, it is possible to calculate the quantity of interest, here the PSD, only as often as desired. It is impossible to give general guidelines for the required overlap, but should the computational cost be of concern, one can make a few tests runs with decreasing overlap periods to observe the decrease in correlations of the subsequent ICs, and thus select an acceptable correlation level and overlap with regard to the signal at hand. To standardize the input signals, after mean removal, each input signal pair was jointly scaled to reside between negative and positive unity. Resulting IC PSD time series are seen in Fig. 9, where also similarly calculated PSD time series are shown for the MFPMs. IC PSD time series provides a method for analyzing evolution of the brain wave frequencies (Levine et. al., 1999; Penttonen and Buzsáki, 2003) present in the FUPOs.

For the IC PSD analysis, it is necessary to determine, which of the ICs from the subsequent ICAs correspond to each other. Here, we used correlation analysis, and determined the corresponding subsequent ICs according to the maximum absolute value of the correlation coefficient. Thereafter, the subsequent ICs were scaled. This can be freely done because of the inherent ambiguity of IC energy. The latter of the two subsequent corresponding ICs was always scaled so that the amplitudes of the subsequent ICs at the time of the maximum of the former were the same, and the sign of the correlation coefficient was used in setting sign of the latter IC. This resulted in a time series of ICs, in which ICs were approximately of the same maximum magnitude, and had the same sign, which allowed observing the ICs throughout the computation to observe the correct identification of corresponding subsequent ICs and possible ICA convergence problems. Note, that scaling the maxima or the means of two subsequent ICs to the same value does not in general yield appropriate scaling of the corresponding subsequent ICs. To strengthen the probability of correlating subsequent ICs, the time window was slid one sample at a time even though every 10th pair of IC PSDs was calculated, and the last found estimate of the mixing matrix \( \hat{\mathbf{A}} \) (7) was always used as the seed in the subsequent ICA.

4. Results of ICA of rat hippocampal FP

In this Section, results of the described ICA calculations are shown, and some exemplary hippocampal FUPO analyses conclusions are drawn to illustrate the proposed analysis method. It is to be noted that the shown examples are too few to truly deduce the workings of the hippocampus, including its frequencies and reverberations. Thus, the results should be considered only as examples demonstrating the proposed FUPO analysis method. In Fig. 5A, the ICs calculated with all combinations of two and three of the signals seen in Fig. 4B as ICA input are shown. Similarly, Figs. 6A, 7A, and 8A, present the ICs calculated from signals seen in Figs. 4C, 4D, and 4E, respectively. PSDs of the ICs in Figs. 5A, 6A, 7A, and 8A, are shown in Figs. 5B, 6B, 7B, and 8B, respectively. In Figs. 5–8, also IC clustering results are shown. Clustering was run separately on each set of nine ICs seen in Figs. 5A, 6A, 7A, and 8A, and the clustering results were copied also onto respective Figs. 5B, 6B, 7B, and 8B.

Let us first take a look at the clustering results without reference to anatomy. With all the combinations of the signals in Fig. 4B as inputs, ICA produced the ICs seen in Fig. 5A, and the clustering grouped the ICs into three clusters. In visual inspection, the clustering of time domain ICs in Fig. 5, agrees fairly well with intuition, both in time domain and in frequency domain. Only IC12 notably differs from the other ICs in cluster 3. Also, observing the frequencies of the PSD peaks in Table 1, it is seen that the PSD peak frequencies within each cluster are fairly similar; PSDs of Cluster 1 ICs contain only one peak at around 3Hz, Cluster 2 PSDs have two peaks, at 0.8 Hz, 3.1 Hz, and 4.5 Hz, and Cluster 3 PSDs have a common peak at a little over 1 Hz. In Fig. 5B, note also the spread activity of the Cluster 2 ICs around 20 Hz, not indicated in Table 1. From the orders of the IC correlation \( p \)-values for Fig. 5A, ICs in the upper left quadrant of Table 3, it is seen that IC11 correlates with IC31 and IC41 with high confidence, i.e., the order of the \( p \)-value is very small. Likewise, IC31 correlates with high confidence with IC41. These correlations agree with the clustering result for Cluster 1. Furthermore, it is seen from Table 3 that the maximum correlation between IC11, IC31, and IC41 is found at zero time lag. In Fig. 5, Cluster 2 is seen to be confirmed by high confidence correlation of IC12 and IC32, whose maximum correlation occurs with zero time lag, as seen from Table 3. ICs from a single ICA should naturally not correlate with each other, but since the found ICs are iteratively calculated approximations, some correlation may remain. In Table 3, the low confidence correlation between Fig. 5 ICs IC21 and IC31, and between Fig. 6 ICs IC42 and IC43, represent such cases.
To deduce the spatial locations of the ICs in Fig. 5A, it is seen that Cluster 1 ICs appear always when the measurement $y_8$ participates in ICA. Referring to Fig. 3, this suggests that Cluster 1 like activity is associated with Sch, with one prominent frequency component at around 3 Hz, c.f., Table 1. Cluster 2 ICs occur with when both measurements $y_6$ and $y_{15}$ participate in ICA, which suggests that CA1 and DG participate in the same principal cell FUPO (CA1/DG FUPO). From Table 1 it is seen that this FUPO carries three prominent frequency components; 0.8 Hz, 3.1 Hz, and 4.5 Hz. From Table 3 it is also seen that Cluster 2 ICs are correlated with high confidence and are in phase with each other. Finally, a Cluster 3 IC is present in all ICAs, i.e., Cluster 3 ICs represent a signal component, which is present in all the considered hippocampal structures, and carries either one or two prominent frequency components; there always is a frequency component at a little over 1 Hz, and in two cases there is also a component at around 3 Hz. From Table 3 it is seen that Cluster 3 is formed with much lower confidence than the other clusters. Furthermore, observing the lower confidence correlations in Table 3, the Cluster 2 ICs, IC 22 and IC 43, are seen to be correlated with low confidence with the Cluster 1 ICs, C 11, C 31, and C 41, with the Cluster 2 ICs preceding the Cluster 1 ICs by 107 ms. This suggests that Sch FUPO activity is related to the CA1/DG FUPO activity, but lags a little over 100 ms behind. There are several possibilities for the delay, rigorous explanation of which would require further investigations, but it may be that either the signal originated from outside the hippocampus, Sch neurites did not fire every time the CA1/DG neurons do, or DG was one cycle ahead of CA1, thus producing a temporal propagation from DG to Sch to CA1.

Table 1. Frequencies (Hz) of the PSD peaks above 10 % of the maximum PSD over all the PSDs in Fig. 5B. The clustering results, seen in Fig. 5, are also shown.

<table>
<thead>
<tr>
<th>PSD(ICA)</th>
<th>Frequency (Hz)</th>
<th>Cluster</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSD(ICA_1)</td>
<td>3.1</td>
<td>Cluster: 1</td>
</tr>
<tr>
<td>PSD(ICA_2)</td>
<td>1.1 3.3</td>
<td>Cluster: 3</td>
</tr>
<tr>
<td>PSD(ICA_3)</td>
<td>1.2 2.7</td>
<td>Cluster: 3</td>
</tr>
<tr>
<td>PSD(ICA_4)</td>
<td>3.1</td>
<td>Cluster: 1</td>
</tr>
<tr>
<td>PSD(ICA_5)</td>
<td>1.3</td>
<td>Cluster: 3</td>
</tr>
<tr>
<td>PSD(ICA_6)</td>
<td>0.8 3.1 4.5</td>
<td>Cluster: 3</td>
</tr>
<tr>
<td>PSD(ICA_7)</td>
<td>1.2</td>
<td>Cluster: 3</td>
</tr>
<tr>
<td>PSD(ICA_8)</td>
<td>0.8 3.1 4.5</td>
<td>Cluster: 2</td>
</tr>
</tbody>
</table>
Fig. 6. (A) ICAs of the sets of two to three signals seen in Fig. 4C, and (B) the PSDs of the ICs in (A). See Fig. 5 for details.

Table 2. Frequencies (Hz) of the PSD peaks above 10% of the maximum PSD over all the PSDs in Fig. 6B. The clustering results, seen in Fig. 6, are also shown.

<table>
<thead>
<tr>
<th>PSD(IC_{11})</th>
<th>PSD(IC_{12})</th>
<th>PSD(IC_{13})</th>
<th>PSD(IC_{41})</th>
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<td>1.0</td>
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</tr>
<tr>
<td>Cluster: 1</td>
<td>Cluster: 2</td>
<td>Cluster: 1</td>
<td>Cluster: 2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PSD(IC_{21})</th>
<th>PSD(IC_{22})</th>
<th>PSD(IC_{23})</th>
<th>PSD(IC_{31})</th>
<th>PSD(IC_{32})</th>
<th>PSD(IC_{42})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.4</td>
<td>2.7</td>
<td>0.8</td>
<td>2.7</td>
<td>0.8</td>
<td>2.7</td>
</tr>
<tr>
<td>3.4</td>
<td>26.5</td>
<td>26.4</td>
<td>26.4</td>
<td>3.2</td>
<td>26.4</td>
</tr>
<tr>
<td>Cluster: 2</td>
<td>Cluster: 1</td>
<td>Cluster: 1</td>
<td>Cluster: 2</td>
<td>Cluster: 1</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>PSD(IC_{43})</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.6</td>
</tr>
<tr>
<td>Cluster: 1</td>
</tr>
</tbody>
</table>

Fig. 6 shows the ICs and their PSDs for 1 s of measurements immediately following those seen in Fig. 5. Again, visual inspection of the ICs and their PSDs fairly well agrees with the clustering results seen in Fig. 6, although one might expect three clusters instead of only two found; IC_{22} and IC_{43} might have been assigned into a cluster of their own, but this is highly subjective. Also, from Table 2 it is seen that IC_{22} and IC_{43} in Cluster 1, and IC_{32} in Cluster 2, exhibit a PSD peak at around 26 Hz. This might also suggest that these three ICs could have formed a third cluster, if some other clustering cutoff point, or distance metric had been used.

Correlation p-values in the lower right-hand quadrant of Table 3 confirm the clustering results seen in Fig. 6. The only discrepancy is the correlation between IC_{31} and IC_{41}, which ICs are assigned to different clusters but are seen to correlate, although the confidence of the correlation is low. From the lower left-hand quadrant of Table 3, it is seen that IC_{11}, IC_{31}, and IC_{41} belong to Cluster 1 with higher confidence than IC_{22} and IC_{43}. Cluster 1 co-occurs with y_6, although there is a Cluster 1 IC, IC_{22} in ICA(y_6,y_15), and IC_{22} correlates with a lower confidence with IC_{11} and IC_{42}, but with a bit higher confidence with IC_{31} and IC_{43}. Cluster 2 is present in all ICAs, but IC_{32} has more high frequency components than the other Cluster 2 ICs, IC_{12}, IC_{21}, or IC_{41}. Although the situation seen in Fig. 6 is not as clear as that in Fig. 5, we may again conclude that Cluster 1 like activity could be associated with Sch FUPO, and Cluster 2 like activity with CA1/DG FUPO. The 26.5 Hz frequency component always co-occurs with the ICA input signal y_{15}, which suggests that at the DG end of the CA1/DG FUPO carries a higher frequency information flow or FUPO than that present at the CA1 end. The CA1 end is probably better illustrated by the Cluster 2 ICs IC_{12} or IC_{21}, for example. From Table 3, it is seen that the ICs, which correlate with the highest confidences have their maximum correlations with near zero time lag, except that IC_{22} lags 25 ms behind IC_{31}.
Table 3. Time lags of maximum correlations in milliseconds (upper values), and the orders of correlation p–values (lower values) for the pairs of ICs in Figs. 5A and 6A, whose p < 0.0001. The smaller the order of the p-value, the stronger the correlation between the two ICs. For example, the strongest correlation between IC41 and IC43 in Fig. 5A is observed when the IC41 lags 107 ms behind IC43. Time lag information is not relevant between the ICs calculated on different time periods; thus, only the orders of p-values are shown in the upper right quadrant of the table for correlations between ICs in Figs. 5 and 6. Observe the note on p-values at the end of Section 3.4.2.

Based on the upper right-hand quadrant of Table 3, ICs which most likely correspond to each others in Figs. 5A and 6A for each ICA with inputs taken from the same multielectrode channels are collected into Table 4. For example, Fig. 5A IC11 is seen to correlate with Fig. 6A IC11 with high confidence, whereas it does not correlate with Fig. 6A IC12. Also, Fig. 5A IC12 correlates with Fig. 6A IC12 with fairly high confidence, while it does not correlate with Fig 6A IC11. Thus, the Fig. 5A IC11 can be taken to represent the same FUPO or information flow as the Fig. 6A IC11, and likewise Fig. 5A IC12 is associated with Fig. 6A IC12, thus allowing following FUPOs in time. For example, observing PSD peaks of Cluster 1 ICs in Tables 2, 3, except for PSD(IC22), and the IC correspondences in Table 4, it can be concluded that Sch has a continued frequency component at around 3 Hz during both time periods of Fig. 5A and Fig. 6A, and a possible 1 Hz component, which has occurred during the latter 1 s period, Fig. 6A, only. Note, that since the considered time period is one second, analyzing very low frequency components, like the 1 Hz component above, may not be reliable. Also, note that Fig. 6A IC43 was not associated with any Fig. 5A ICs, because only the maximum confidence correlations are shown in Table 4. Also, it would have
been possible to run clustering with all the 18 ICs seen in both Fig. 5A and 6A, thus yielding an IC grouping over the subsequent time periods. Similar information can also be deduced from the upper right quadrant of Table 3. For example, starting with the Cluster 1 ICs of Fig. 5A, and setting, here arbitrarily, the maximum allowable order of correlation $p$-value to $10^{-20}$, yields the following group of similar ICs: Fig. 5A: IC11, IC31, and IC41, and Fig 6A: IC11, IC31, and IC42, which six ICs together could form a single cluster. Although this method allows us to analyze the FUPOs at a point in time, or rather to summarize the FUPOs during a short period of time at one glance, observing the IC correspondences concerning the three-input ICAs in Table 4, it is seen that the analysis did not provide one-to-one correspondences between ICs from subsequent time periods, thus making it difficult to address time evolution of FUPOs. This is due to the dynamic nature of FUPOs. The FUPO time evolution is made visible by the sliding window method, described in Section 3.4.3, and illustrated in 4.1., where ICA is calculated every 1/1250 s, instead of once per second.

Table 4. ICs related to each other between ICAs in Figs. 5A and 6A, based on the maximum correlation $p$-values in the upper right-hand quadrant of Table 3. E.g., IC41 in Fig. 5A correlates with high confidence with IC42 in Fig. 6A.

<table>
<thead>
<tr>
<th>ICA($y_6,y_8$)</th>
<th>ICA($y_6,y_{15}$)</th>
<th>ICA($y_8,y_{15}$)</th>
<th>ICA($y_6,y_8,y_{15}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fig. 5A ICs</td>
<td>Fig. 6A ICs</td>
<td>Fig. 5A ICs</td>
<td>Fig. 6A ICs</td>
</tr>
<tr>
<td>IC11</td>
<td>IC11</td>
<td>IC12</td>
<td>IC12</td>
</tr>
<tr>
<td>IC12</td>
<td>IC12</td>
<td>IC22(*)</td>
<td>IC22(*)</td>
</tr>
<tr>
<td>IC31</td>
<td>IC31</td>
<td>IC32(*)</td>
<td>IC32(*)</td>
</tr>
<tr>
<td>IC41</td>
<td>IC42</td>
<td>IC42(*)</td>
<td>IC41(*)</td>
</tr>
<tr>
<td>IC43</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

*) Correlation $p$-value is relatively very high, i.e., correlation is of low confidence.

Table 5. Frequencies (Hz) of the PSD peaks above 10% of the maximum PSD over all the PSDs in Fig. 7B. The clustering results, seen in Fig. 7, are also shown.

<table>
<thead>
<tr>
<th>PSD(IC11): 0.9</th>
<th>PSD(IC12): 12.9 14.0 15.1 28.0</th>
<th>PSD(IC13): 0.8 12.9 14.0 28.0</th>
<th>PSD(IC14): 0.8 13.0 14.0 28.0</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cluster: 2</td>
<td>Cluster: 5</td>
<td>Cluster: 5</td>
<td>Cluster: 5</td>
</tr>
<tr>
<td>PSD(IC12): 1.0 12.9 14.0</td>
<td>PSD(IC12): 1.1 13.0 14.0</td>
<td>PSD(IC12): 1.1 13.0 14.0</td>
<td>PSD(IC12): 1.1 3.4 14.0 28.0</td>
</tr>
<tr>
<td>Cluster: 1</td>
<td>Cluster: 1</td>
<td>Cluster: 3</td>
<td>Cluster: 4</td>
</tr>
<tr>
<td>PSD(IC43): 0.8 14.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cluster: 3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

ICs of the signals seen in Fig. 4D during the electrical stimulation period are seen in Fig. 7, along with their PSDs. Table 5 lists the frequencies of the PSD peaks reaching over 10% of the maximum PSD value over all the PSDs in Fig. 7B. Clustering seen in Fig. 7, agrees with visual inspection of the PSDs in Fig. 7B; the ICs in each cluster are seen to carry their particular
The whole hippocampus with the frequency a little over 1 Hz, except for IC43, which has a minor PSD peak around 5 Hz.

The considered hippocampus, and consistently carry a brain wave frequency at 0.8 Hz. Cluster 1 like activity also spans of Fig. 8A, frequency domain behavior seen in Fig. 8B, and PSD peak frequencies seen in Table 6. Cluster 2 like signals span both are present in the whole hippocampus. In a case like seen for ICA(

Judging from Fig. 8, based on both the time domain signal shapes and PSDs, IC11, and IC43, and possibly IC32, could have been assigned to a cluster of their own since they contain mostly noise. Assuming these three clusters, one possible conclusion is that in the Fig. 8, the Cluster 2 like signals span the whole hippocampus, IC22 and IC42 represent the signal present in the CA1/ DG FUPO, and finally IC11, IC43, and possibly IC32, carry mostly noise, which co-occurs with the channel y8, i.e., with the Sch FUPO. Given the clustering in Fig. 8, the conclusion would be that the hippocampus exhibits only two FUPOs, which both are present in the whole hippocampus. In a case like seen for ICA(y6, y8, y15) in Fig. 8, noise reduction by PC elimination before ICA could have been used.

During the stimulation, clustering groups the ICs in Fig. 7A into five clusters, whereas after the stimulation only two clusters of ICs are found, c.f., Fig. 8A. Had the electrical stimulation caused an electrical breakdown of the hippocampal tissue, it would have been expected that during the stimulation, only one IC cluster exhibiting the stimulation signal had been found, while the rest of the ICs would have been composed of noise. Now, on the contrary, from the high number of clusters in Fig. 7, it can be concluded that a high number of hippocampal FUPOs have responded to the stimulation, each in its own way. Thus, the electrical stimulation can be deemed to have had a true stimulation effect. After the stimulation, hippocampal activity exhibits only one clear FUPO, which is represented by the Cluster 2 ICs in Fig. 8. Cluster 1 ICs in Fig. 8, especially IC11, IC32, and IC43, exhibit mostly noise or incoherent intrinsic activity of mostly individual hippocampal neurons.

Compared to the previous cases; normal brain operation seen in Figs. 4B and 4C, and especially to the stimulated condition in Fig. 4D, the situation during the time right after electrical stimulation in Fig. 4E presents us with the other extreme. It is seen from the ICs, their PSDs, and from the clustering results in Fig. 8, and also from Table 6, that now the whole hippocampus exhibits only two FUPOs or information flows. Again, clustering groups similar signals well, according to the visual inspection of Fig. 8A, frequency domain behavior seen in Fig. 8B, and PSD peak frequencies seen in Table 6. Cluster 2 like signals span the whole considered hippocampus, and consistently carry a brain wave frequency at 0.8 Hz. Cluster 1 like activity also spans of Fig. 8, frequency domain behavior seen in Fig. 8B, and PSD peak frequencies seen in Table 6. Cluster 2 like signals span the whole hippocampus, IC22 and IC42 represent the signal present in the CA1/DG FUPO, and finally IC11, IC43, and possibly IC32, carry mostly noise, which co-occurs with the channel y8, i.e., with the Sch FUPO. Given the clustering in Fig. 8, the conclusion would be that the hippocampus exhibits only two FUPOs, which both are present in the whole hippocampus. In a case like seen for ICA(y6, y8, y15) in Fig. 8, noise reduction by PC elimination before ICA could have been used.

During the stimulation, clustering groups the ICs in Fig. 7A into five clusters, whereas after the stimulation only two clusters of ICs are found, c.f., Fig. 8A. Had the electrical stimulation caused an electrical breakdown of the hippocampal tissue, it would have been expected that during the stimulation, only one IC cluster exhibiting the stimulation signal had been found, while the rest of the ICs would have been composed of noise. Now, on the contrary, from the high number of clusters in Fig. 7, it can be concluded that a high number of hippocampal FUPOs have responded to the stimulation, each in its own way. Thus, the electrical stimulation can be deemed to have had a true stimulation effect. After the stimulation, hippocampal activity exhibits only one clear FUPO, which is represented by the Cluster 2 ICs in Fig. 8. Cluster 1 ICs in Fig. 8, especially IC11, IC32, and IC43, exhibit mostly noise or incoherent intrinsic activity of mostly individual hippocampal neurons.

Fig. 8. (A) ICAs of the sets of two or three signals seen in Fig. 4E, and (B) the PSDs of the ICs in (A). See Fig. 5 for details.

Table 6. Frequencies (Hz) of the PSD peaks above 10 % of the maximum PSD over all the PSDs in Fig. 8B. The clustering results, seen in Fig. 8, are also shown.

<table>
<thead>
<tr>
<th>PSD(IC11)</th>
<th>PSD(IC22)</th>
<th>PSD(IC31)</th>
<th>PSD(IC41)</th>
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<tr>
<td>1.1</td>
<td>0.8</td>
<td>0.8</td>
<td>0.8</td>
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<tr>
<td>Cluster: 1</td>
<td>Cluster: 2</td>
<td>Cluster: 1</td>
<td>Cluster: 2</td>
</tr>
<tr>
<td>PSD(IC12)</td>
<td>PSD(IC23)</td>
<td>PSD(IC32)</td>
<td>PSD(IC42)</td>
</tr>
<tr>
<td>0.8</td>
<td>1.1</td>
<td>1.2</td>
<td>1.1</td>
</tr>
<tr>
<td>Cluster: 2</td>
<td>Cluster: 1</td>
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<tr>
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<td>PSD(IC43)</td>
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<tr>
<td>4.9</td>
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<tr>
<td>Cluster: 1</td>
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</table>
4.1. FUPO time evolution

To demonstrate FUPO PSD time evolution calculations, we used the total input signal length of 2 s, and the results are presented in Fig. 9. ICA in a sliding window, and the PSDs, were calculated as described in Section 3.4.3. on all pairs of the three 2 s MFPM signals seen in 1 s sections in Figs. 4B and 4C. Results from ICA(\(y_6,y_8,y_{15}\)) are omitted but can be obtained exactly similarly. In Figs. 9A, 9B, and 9C, are seen the PSD time series for the ICA input signals \(y_6, y_8\) and \(y_{15}\), respectively. Figs. 9D and 9E show the PSDs of the ICs from ICA(\(y_6,y_8\)), Figs. 9F and 9G PSDs of ICs from ICA(\(y_6,y_{15}\)), and Figs. 9H and 9I PSDs of ICs from ICA(\(y_8,y_{15}\)). In Fig. 9F, PSDs are placed on the time axis according to the mid time points of the signals used in their calculations with respect to the time scale of Figs. 4B and 5A.

PSDs at time 0.5 s in Figs. 9D–I correspond to the PSDs of ICs from the two-input ICAs seen in Fig. 5B, and at time 1.5 s to those in Fig. 6B. At time 0.5 s, PSD(\(IC_1\)) of ICA(\(y_6,y_8\)) in Fig. 9D corresponds to PSD(\(IC_{11}\)) in Fig. 5B, and PSD(\(IC_2\)) of ICA(\(y_6,y_8\)) in Fig. 9E to PSD(\(IC_{12}\)) in Fig. 5B. Similarly, at time 0.5 s, PSD(\(IC_1\)) of ICA(\(y_6,y_{15}\)) in Fig. 9F corresponds to PSD(\(IC_{21}\)) in Fig. 5B, PSD(\(IC_2\)) of ICA(\(y_6,y_{15}\)) in Fig. 9G to PSD(\(IC_{22}\)) in Fig. 5B; PSD(\(IC_1\)) of ICA(\(y_8,y_{15}\)) in Fig. 9H to PSD(\(IC_{31}\)) in Fig. 5B; and PSD(\(IC_2\)) of ICA(\(y_8,y_{15}\)) in Fig. 9I to PSD(\(IC_{32}\)) in Fig. 5B. Similar mapping can be observed between the PSDs at time 1.5 s in Figs. 9D–I, and the PSDs of the ICs from the two-input ICAs in Fig. 6B. In Fig. 9I, notice especially the disappearance of the prominent frequency component at 1.3 Hz, c.f. Table 1, seen in PSD(\(IC_{32}\)) in Fig. 5B, but has vanished by the time of Fig. 6B PSD(\(IC_{32}\)).

Comparing Figs. 9A and 9B to Figs. 9D and 9E, it is seen that the major frequency components are present both in the measurements and in the ICs, but that ICA has redistributed the frequency components into the ICs; e.g., approximately during the time between 0.5 s and 0.9 s, PSD(\(IC_2\)) in Fig. 9E lacks the frequency components above 4 Hz, which are present in both ICA input signals input \(y_6\) and \(y_8\) in Figs. 9A and 9B, respectively. PSDs of ICs from ICA(\(y_6,y_8\)) in Figs. 9D and 9E show clear abrupt changes in the IC frequency content at a little before 1 s time point. Such a transition is either due to a sudden change in the frequency pattern of the FUPOs, or subsequent ICAs converging to clearly differing IC pairs. Also, one must take care that the subsequent corresponding ICs are correctly identified, since the order of the ICs is indeterminate. In such case, the time evolution seen in the PSD of one IC would be seen to resume in the PSD of the other IC after a sharp transition; here, this is clearly not the case. To investigate further, we observed the individual ICs throughout the calculations while using all the available FastICA nonlinearity functions, and also with double sampling rate. An abrupt change in the ICs was always observed, and it was confirmed not to be a mere interchange of the ICs. Also the PSD plots still exhibited similar abrupt changes as seen in Figs. 9D and 9E. The only exception was that with the nonlinearity function ‘skew’, FastICA completely failed to produce similar subsequent ICs. Calculating the ICs using half of the original sampling rate resulted in no abrupt changes in the subsequent ICs and also the PSD plots did not exhibit any sudden changes (data not shown). Thus, it can be concluded that the abrupt change in Figs. 9D and 9E is due to the subsequent FastICA calculations converging to different solutions. It is worth noting that the abrupt change seen in Figs. 9D and 9E is much less severe than what one would expect when observing the rather severe change in the corresponding subsequent ICs. Furthermore, should such a sudden change in brain wave frequencies be due to physiological factors, one could expect the change to be at least somewhat visible also in some of the ICs from other ICAs, and not to vanish with changing calculation parameters.

Looking at the PSD(\(IC_1\)) and PSD(\(IC_2\)) in Figs. 9F and 9G, respectively, and comparing them with the corresponding PSDs of the ICA input signals seen in Figs. 9A and 9C, it is seen that \(IC_1\), Fig. 9F, has inherited the most prominent silent time-frequency area above 4 Hz of the input signals. Also, \(IC_1\) is seen to carry more distinct frequency bands at approximately 8 Hz, 12 Hz, and 15 Hz, although these PSD peaks are too low to appear in Tables 1 and 2. On the other hand, \(IC_2\) in Fig. 9G, is seen to be cluttered with numerous frequency components throughout the depicted time-frequency area. More interestingly, \(y_8\) and \(y_{15}\) carry common main features, which are captured by PSD(\(IC_1\)) in Fig. 9F, whereas into PSD(\(IC_2\)), Fig. 9G, ICA has dug out a frequency evolution structure, which resembles that seen for the input signal \(y_8\), especially below 4 Hz after 0.9 s. \(y_8\) like signal may thus be present also in either or both of the measurements \(y_6\) and \(y_{15}\), although this is not evident only by observing the PSDs of these measurements.

Comparing PSD(\(IC_1\)) and PSD(\(IC_2\)) in Figs. 9H and 9I, respectively, with the input signal PSDs in Figs. 9B and 9C, the time-frequency structure of \(IC_1\), in Fig. 9H is found fairly similar to that of the input signal \(y_8\) in Fig. 9B. Similarly, PSD(\(IC_2\)) in Fig. 9I, resembles the PSD(\(y_{15}\)) in Fig. 9C. Correlation analysis of the input signals would probably reveal that already the two input signals were fairly independent, although, note the weakening of the distinct low frequency component in Fig. 9F, as already mentioned.
Fig. 9. (A) PSD time series of $y_6$, (B) of $y_8$, and (C) of $y_{15}$, during the time period seen in Figs. 4B and 4C. (D)–(I) show the PSD time series of the ICs from the ICA calculations as stated in the subfigures.
Fig. 9. (Continued).

Drawing from the earlier conclusions on the relations between the hippocampal structures and the ICs, it can be concluded that the PSD time series seen in Figs. 9D and 9H represent frequency component time evolution of the Sch FUPO, since these PSD time series correspond to the Cluster 1 ICs in Fig. 5. Indeed, the PSD time series in Figs. 9D and 9H do share interesting time-frequency features; especially, the evolution of frequency bands below 7 Hz is distinctively similar. For example, the weak frequency component at a little above 5 Hz apparent at time 0.5 s raises steadily up to 6 Hz or 7 Hz at 1.2 s. Also, the main frequency component around 3 Hz at 0.5 s first splits to yield a 4 Hz frequency at time 0.7 s, which splits again a little after 1 s time point to produce a new component at a little over 5 Hz.

IC PSD time evolution seen in Fig. 9G corresponds to the Cluster 2 like activity in Fig. 5, which was associated with the CA1/DG FUPO. At frequencies below 4 Hz, the frequency component time evolution structure looks fairly well alike that described for the Sch FUPO, except that the frequency components are of more equal strength, while for Sch FUPO, the 3 Hz component was distinctively stronger than the other components. As already mentioned, at frequencies above 4 Hz, the time-frequency representation of CA1/DG FUPO in Fig. 9G is more densely filled with frequency components than that of the Sch FUPO. Also, the CA1/DG FUPO PSD time evolution is missing the frequency components whose frequencies are ramping through frequencies, like seen for the Sch FUPOs, e.g., in Fig. 9H.

IC PSD time evolutions seen in Figs. 9E, 9F, and 9I can be associated with the Cluster 3 ICs in Fig. 5, and can thus be taken to represent processes, which are global to the hippocampal structures considered. In Figs. 9E, 9F, and 9I, a common frequency component at around 1 Hz or 2 Hz is evident. Also, the most prominent silent parts of the three time-frequency presentations show some resemblance to each other.
5. Discussion

In this paper, we have described a method for analyzing neural populations from multielectrode FP measurements using ICA. The proposed ICA based FUPO analysis method provides new means for analyzing the function of neural networks, and may be applied to any multielectrode measurement, for which the basic ICA assumptions are satisfied at least to a degree that the ICA algorithm will converge to an acceptable solution. The requirement for applying ICA is that there exist a number of simultaneous measurements, which carry linear combinations of the original source signals. For FUPO analysis, this means that one has to record at least a few mixtures of FUPO FPs simultaneously; a requirement, which is generally well satisfied by most multielectrode measurements, in which the distance between MPs is sufficiently small. It is also required that the original sources are nongaussian. While it is impossible to check this beforehand, FastICA strives to iterative maximize nongaussianity, and thus, if sufficiently good approximations of ICs are found, they are nongaussian. Although, we also encountered MFPMS for which we could not get FastICA to converge. Such measurements with the selected sampling rate and frequency band, probably did not fulfill the assumptions of ICA. While it is generally not possible to determine the actual number of FUPOs present in only a few concurrent measurements, it is possible to include more measurements into ICA, until hopefully no more information bearing ICs are found. Also, although not demonstrated in this paper, it may be possible to subject the found similar ICs from separate ICA calculations, e.g. the ICs grouped to one cluster, again to ICA, thus possibly revealing further ICs.

We first demonstrated the basic ICA functioning with a simulation, and then applied the proposed ICA analysis to real multielectrode FP measurements from the rat hippocampus. The proposed analysis was shown to yield ICs, which can be analyzed relative to each other to yield FUPO position information relative to the MPs. In our example, three input signals were used to allow illustrative demonstration of the proposed analysis method, and in the case of normal hippocampal function, for example, three FUPOs were identified; 1) DG/CA1 FUPO corresponding to cell somas, 2) Sch FUPO corresponding to neurites, and 3) an universal FUPO extending through the whole hippocampus seen by the multielectrode measurement. To fully analyze the functioning of the brain structure of interest, the full set of available multielectrode measurements could have been included in the analysis. Since our aim was to demonstrate the ICA of multielectrode measurements, in our opinion three physiologically most relevant measurement channels were selected as ICA inputs. This was possible, since in the hippocampus, it was possible to associate the multielectrode measurement channels with the cellular layers based on the known features of the measured signals. Clearly, for some brain structures, and especially if all the simultaneous measurements were made from a single cellular layer, this may not be possible. In such a case, the analysis can be best approached by including all the available measurement channels, a suitably selected subset a time, into ICA. If single cell recordings, recorded simultaneously with the multielectrode recordings from the same target structure, were available, these could be used to associate an IC with a specific FUPO, and to pinpoint its location, thus providing increased level of accuracy. Here, the FUPOs and their relative locations were deduced from the ICs from ICAs using all combinations of two and three channels of the selected three multielectrode measurement channels.

The ICs were analyzed for frequency content, and to find similar ICs from different ICAs, the ICs were clustered, and on the other hand, their correlation p-values were determined, and the time lags of the maximum correlations were recorded. The correlation time lag analysis might also be conducted separately within each major frequency component seen as peaks in the PSDs. This could yield more conclusive information on relative phases of information flow between the identified FUPOs. Also, it is possible to first find the maximum correlation time lags of the measurements, and perform ICA on accordingly time shifted FP measurements. Doing so might yield more prominent ICs, but might also obscure some minor ICs, which might have been visible otherwise. To most completely analyze the data, it would be possible to perform ICA on all combinations of available simultaneous FP measurements with all physiologically relevant time shifts between the measurements channels. Since ICA assumes that an IC is in the same phase simultaneously in all the measurements, it is possible to include more measurements into ICA, until hopefully no more information bearing ICs are found. Also, although not demonstrated in this paper, it may be possible to subject the found similar ICs from separate ICA calculations, e.g. the ICs grouped to one cluster, again to ICA, thus possibly revealing further ICs.

To extend the analysis described in this paper, both simultaneous intracellular and extracellular multielectrode measurements can naturally be analyzed together using ICA to enhance the analysis. Intracellular measurements yield exactly localized activity information directly, but they alone cannot provide much information on the workings of neurons with regard to their surroundings. Correlation of the FUPOs with the intracellular measurements could provide exact single FUPO or information flow verification. FUPO-intracellular measurement correlation also allows observing a single neuron function in relation to several active FUPOs in its vicinity. The intracellular measurement may simply be presented to ICA like any one channel of the multielectrode FP measurement.

It is to be noted that one must carefully relate the anatomy of the neural structure to the ICA findings in order to draw correct conclusions about the FUPOs or the information flows present, in order to be able to deduce the functioning of the target brain structure. Without carefully considering neuroanatomy, one can only deduce that the observed ICs are present, but they reveal little about the functioning of the measured brain structure. On the other hand, from the information processing and information–theoretic analysis (Rolls and Treves, 1997; Neelakanta, 1999) point of views, also such ICs might be interesting by the virtue of their existence. Regardless of whether the found ICs are interpretable as FUPOs or information flows, they may yield information on the information coding, flow and processing.
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References


