MASTER 1

Internship report

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Detection of cortical UP and Down states from Local Field Potential

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"I attested that I have read and validated this report"

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(Joana Lourenço)

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Abstract:

The cerebral cortex is the outermost wrinkled part of the brain and it is involved in many cognitive processes including sensory information processing and decision making. It is composed of excitatory and inhibitory neurons, which intertwines each other to make neural circuits. Neural circuits interconnect to one another to form large scale brain networks and contribute to a variety of brain states, each characterized by distinctive spatiotemporal patterns of rhythmic activity and connectivity, depending on the behavioral status. Therefore, neuronal circuits generate brain oscillations, or brainwaves, which have been postulated to be underlying several cognitive functions. During Non-Rapid Eye Movement sleep and anesthesia, the cumulative neuronal activity from cortical areas is dominated by activity of low frequency but high amplitude. Whereas during awake state, it is dominated by an activity of high frequency and low amplitude. Cumulative neuronal activity measured by Electroencephalogram or Local Field Potential during anesthesia and Non-Rapid Eye Movement manifest slow switching between periods of activity and silence of neural networks. Therefore, in order to understand neural networks' dynamics and different cortical processes like synaptic plasticity, sensory integration etc. during these alternating periods of activity and silence, detection of these alternating periods is imperative. To this aim, we measured local field potential simultaneously with whole cell intracellular and loose-patch juxtacellular recording from prefrontal cortex of anesthetized mice. To detect the periods of activity and silence, we implemented two approaches. First one, based on the differential spectral composition of the local field potential in the beta/gamma frequency band (20-100) and second one based on the phase of low frequencies (<7 Hz) of local field potential considering whole cell intracellular recording as ground truth for network state detection. By implementing these two methods, we were able to precisely detect the alternating states of activity and silence from local field potential recorded from prefrontal cortex of anesthetized mice. Our analysis was also able to calculate the average time period of a state, total number of a state, power spectral density ratio of active and silent state. Furthermore, we analyzed firing characteristics of neurons in periods of activity and silence using simultaneously recorded loose-patch juxtacellular recording. In conclusion, detection of periods of silence and activity will be helpful in study of several cortical processes during alternating periods of activity and silence and further will allow investigation of cognitive impairments

List of figures

List of abbreviations, acronyms and notations

Abbreviation/Acronym/notation	**Full Term**
LFP	Local Field Potential
EEG	Electroencephalograph
PFC	Prefrontal Cortex
Vm	Membrane Potential
HEPES	(2-hydroxyethyl)-1-piperazineethanesulfonic
aCSF	artificial Cerebrospinal Fluid
FFT	Fast Fourier Transformation
IFFT	Inverse Fast Fourier Transformation
PSD	Power Spectral Density
S_comb	Combined evidence variable
ROC	Receiver Operator Characteristic
SS Cortex	Somatosensory Cortex
$\Phi_X(t)$	phase at time t in a bandwidth x
$k_X(t)$	power at time t in bandwidth x
$L_x(t)$	Differential liklihood function of band x
$S_{LFP}(t)$	Evidence variable
θ	Angle for sine wave fitting to $L_x(t)$
Std	Standard deviation
EGTA	Egtazic Acid
MEA	Multi Electrode Array

Introduction:

The cerebral cortex is the outermost, typically with the thickness of 2-3 mm, of gray matter over the hemispheres. It is involved in many complex processes such as decision making, sensory information processing and integration, motor control and higher cognitive functions. Classically the structural organization of the cerebral cortex has been based on the key concept of cortical tissue division into different regions, areas, or subareas, where each unit can be typically delineated using cytoarchitectonic histology. The sensory cortex of the mouse is an amenable model in research as it allows to relate to the structural organization of cortex to specific neural activity and possibly function. In their natural environment, humans and animals receive multimodal sensory stimuli. The sensation of these external stimuli is relayed to certain structures of the central nervous system before it enters the cortex of the brain. The first cortical regions which receive the input from the outer world are the primary sensory cortices, such as the primary visual cortex (V1), the primary auditory cortex (A1), or the primary somatosensory cortex (S1). Generally, these regions are anatomically separated. Directly upstream of the primary areas are the secondary cortices, which is often called an associative cortex (Kennedy, Dehay, & Bullier, 1986). The label "associative" can refer to the association of various features of stimuli, but convincing evidence rather points for a multimodal interplay already at the level of the primary sensory cortices (Kayser, 2010). All cortical areas are composed of several intertwined networks of excitatory and inhibitory neurons where excitation is generated by glutamatergic principle neurons and inhibition by GABAergic interneurons. During information processing, interaction of these networks give rise to a complex spatiotemporal activity.

During different behavioral conditions (walking, watching, thinking, sleeping etc.) encountered in a day, cortical activity switches between different brain states, each characterized by a specific spatiotemporal pattern of activity. Mainly, brain switch between two states : "synchronized" and "desynchronized". Desynchronized brain state is related to the vigilant or active behavior, which involves low amplitude but high frequencies in Electroencephalogram (EEG) recording, Local Field Potential (LFP) and neuronal membrane potential (Vm). Conversely in synchronized state, which mainly results from some types of anesthesia and quiet wakefulness, LFP and EEG measurements contain signals of low frequency (slow changes in signal level) of large amplitude (Black, 1937),(Antognini & Carstens, 2002). During these slow changes (<1 Hz) in LFP signal level, the simultaneously recorded membrane potential switches between depolarized 'UP' and hyperpolarized 'DOWN' states (figure 1A).

Commonly used anesthetics that induce the "synchronized" state have allowed researchers to investigate the dependence of cortical network dynamics during UP and DOWN states, in a relatively controlled and stable manner. Many researchers have examined several cortical processes ranging from synaptic plasticity (Huber, Ghilardi, Massimini, & Tononi, 2004), network dynamics and sensory integration during Up and Down states. Investigation of network dynamics in healthy animals is helpful in understanding key operating principles of neural networks in information during the periods of activity and silence induced by anesthesia. Subsequently, understanding of neural networks' operating principles will allow us understand cognitive impairments, such as Down syndrome, autism spectrum disorder and schizophrenia, better.

Previously, most of the experiments relied on challenging in vivo intracellular recordings to detect the Up and Down states of a network by measuring the changes in membrane potential (Timofeev, Grenier, & Steriade, 2001). But as Up and Down states are a network phenomenon, we need an alternate method (to intracellular) to detect these alternating states from a network activity measurement like LFP (Steriade et al., 2011).

Intracellular activity recorded from a neuron by using patch clamp shows alternating periods of depolarization and hyperpolarization (figure 1A). Distribution of recorded intracellular signal (membrane potential) indicates even clearer bimodality, switching between hyperpolarized (Down) and depolarized (Up) states. Up and Down states can then be easily detected by applying a threshold, calculated from the statistics of bimodal distribution of membrane potential, in recorded intracellular activity. However, it is not possible to detect Up and Down states by applying a threshold to the LFP signal because LFP signal doesn't show bimodality (figure 1B).

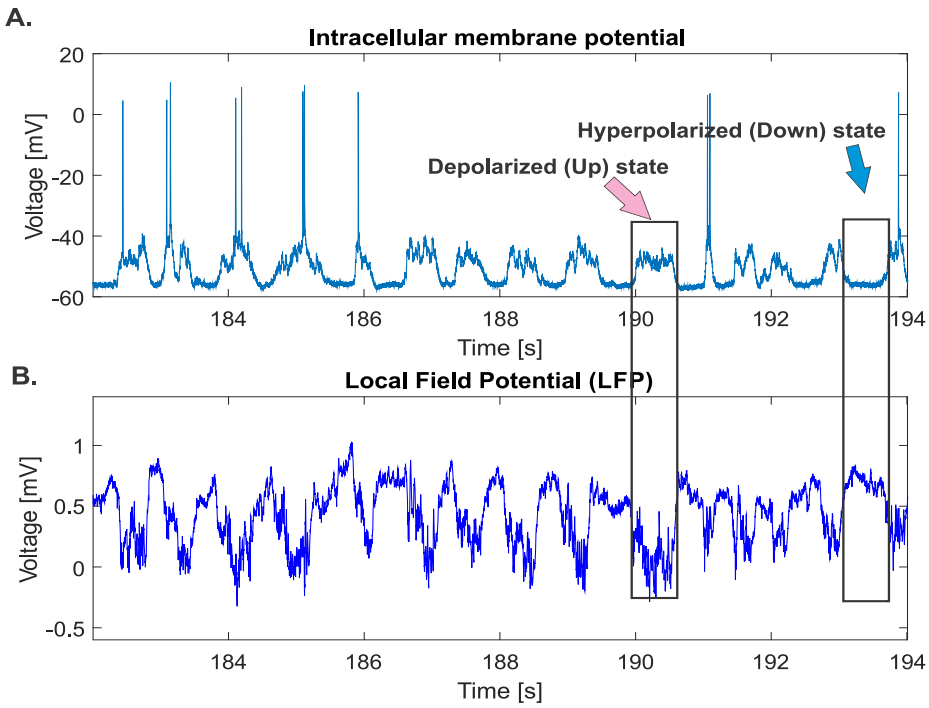

Figure1: Periods of activity and silence (Up and Down states) in whole cell intracellular and Local Field Potential (LFP). A) Bistability shown in whole-cell intracellular recording, alternating periods of hyperpolarization and depolarization (represented by arrows). B) Local Field Potential showing switching between Up and Down states and LFP signal corresponding to depolarized and hyperpolarized states

To detect Up and Down states, we performed LFP (figure 2C), loose-patch juxtacellular recording (figure 2D) and also whole cell intracellular recording (figure 2D) for ground truth of the state detection from prefrontal cortex (PFC) of anesthetized mice and used two methods, Mukovski et al [8] and Saleem et al method [9]. Mukovski et al method uses differential spectral composition during Up and Down states of LFP in beta/gamma

frequency band and Saleem et al uses phases of low frequencies of (<7 Hz) LFP signal, both having whole cell intracellular as ground truth. We show precise detection of Up and Down states from LFP by combining abovementioned methods unlike membrane potential.

1. Experimental Procedure and Methods:

1.1 Experimental Procedure:

The recording configuration used in the experiments is as shown in figure 2A. Mice were anesthetized with 15% urethane (1.5 g/kg in physiological solution) or with ketamine/xylazine (Ketaset, 100 mg/mL/Rompun 20 mg/mL; 10 mL/g body weight) and placed on a stereotaxic apparatus. The body temperature was constantly monitored and kept at 37 °C with a heating blanket. To ensure a deep and constant level of anesthesia, vibrissae movement, eyelid reflex, response to tail, and toe pinching were visually controlled before and during the surgery. An intraperitoneal Rimadyl injection (4 mg/Kg in physiological solution) was performed to reduce and control pain.

A local lidocaine injection was performed over the cranial area of interest and, after a few minutes, a longitudinal incision was performed to expose the skull. Two small cranial windows (<1 mm) were opened at coordinates (2.8 mm from bregma, 0.5 mm lateral to sagittal sinus for PFC; 0 mm bregma, 3.5 mm lateral to sagittal sinus for SS Cortex), while keeping the surface of the brain moist with the normal HEPES-buffered artificial cerebrospinal fluid (aCSF).

To record local field potential (LFP) a borosilicate glass electrode (0.5-1 MΩ resistance) was inserted in the cortex. Electrical signals were acquired and digitized at 20 kHz using a Digidata 1440 interface and PClamp software (Molecular Device, Sunnyvale, CA, USA). A typical LFP recording acquired using these settings can be seen in figure 2C.

Electrophysiological juxtasomal/juxtacellular recordings (figure 2D) were performed with borosilicate glass pipettes (2 mm outer diameter, 5–7 MΩ resistance), which were filled with extracellular solution containing (in mM): NaCl 127, KCl 3.2, CaCl2 2, MgCl2 1, HEPES 10. Pipettes were initially lowered through the pia applying a positive pressure (250 mbar) until the depth of interest was reached. At this point, a lower positive pressure (25 mbar) was applied. When a contact between the pipette and the putative cell was achieved, the positive pressure was released and a dim negative pressure was applied to facilitate the formation of the juxtacellular configuration (resistance up to 40 MΩ). For whole-cell intracellular recordings, Patch pipettes (5-7 MΩ) of 1.5 mm external diameter borosilicate glass (WPI) were pulled using a Narishige P100 Vertica Puller and filled with (in mM): 135 K-gluconate, 6 KCl, 10 HEPES, 1 EGTA, 4 MgATP, Na2ATP and 8 phosphocreatin, pH adjusted to 7.2 with KOH, 290-295 mOsm. Pipette capacitance was neutralized before break-in. High positive pressure (600 mmHg) was applied to the pipette to prevent tip occlusion. Once reached in the specified region of PFC, pressure was reduced until 15 mmHg, the pipette was then advanced in 2-µm steps, and pipette resistance was monitored in the conventional voltage clamp configuration.

When the pipette resistance suddenly increased, positive pressure was relieved to obtain a GΩ seal (small negative pressure can be applied to achieve seal formation).

Seal resistances were always >1 GΩ, and access resistances were typically 30–70 MΩ. Recordings were made in current-clamp mode, and no holding current was applied. Data were acquired using a Multiclamp 700B Amplifier (Molecular Devices).

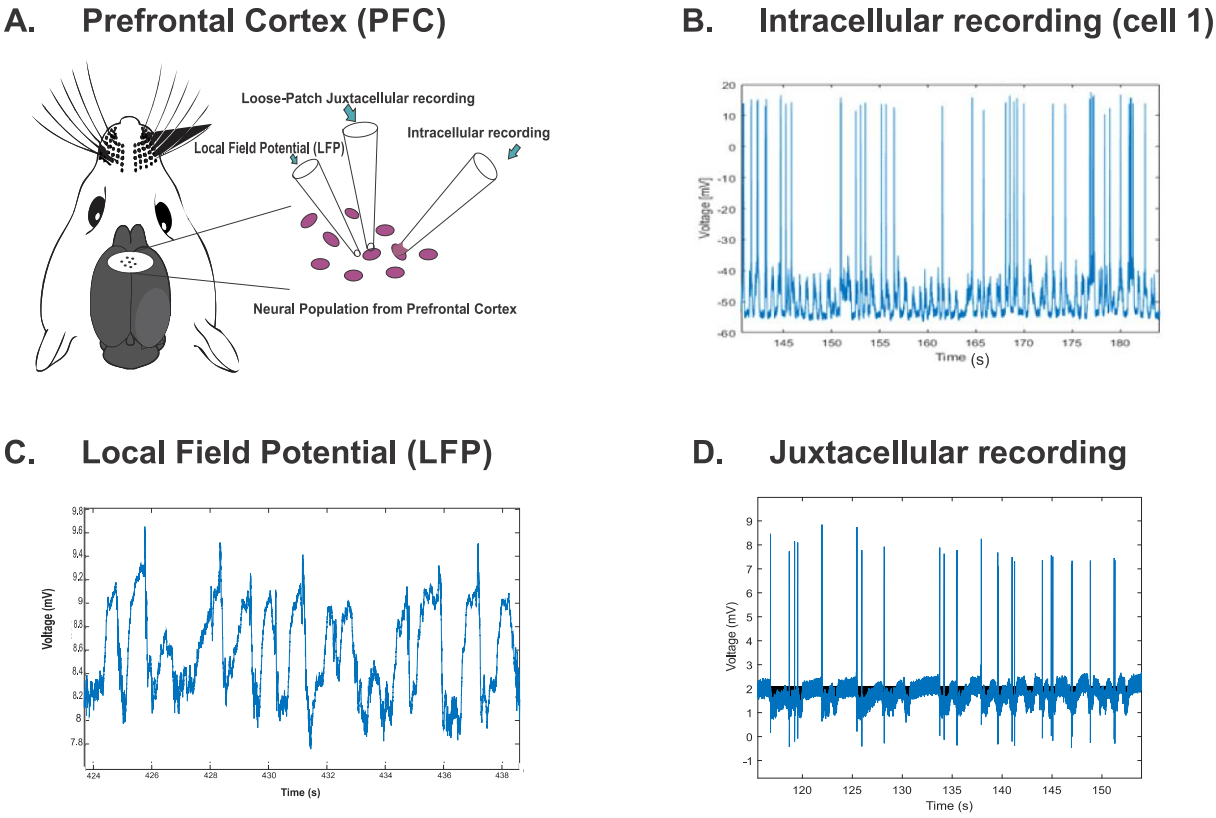

Figure 2 : Recording configuration and typical examples of recordings. A) An illustration of LFP, Intracellular and Juxtacellular recordings from prefrontal cortex. B) A typical example of whole cell Intracellular recording from PFC. C) Simultaneously recorded Local Field Potential (LFP) either with intracellular or Juxtacellular recording. D) An example of loose-patch juxtacellular recording

Detecting cortical UP/DOWN state from membrane potential

Cell membrane potential changes from a depolarized level during an active (UP) state to hyperpolarized level during a silent (DOWN) state and vice versa shown in figure 3A. In the past, this shift in membrane potential has been used to detect cortical UP and DOWN states. First step in the preprocessing of raw intracellular signal is to median filter it in order to remove fast components of signal such as spikes and abrupt change. Then use a bandpass filter with lower cutoff frequency 0.1 Hz so that the signal can be drift corrected (running up or down) and higher cutoff of 20 Hz to make it smooth by removing high frequency components mostly contributed by spikes (Mukovski, Chauvette, Timofeev, & Volgushev, 2007). The processed signal is shown in figure 3B where slow alternating periods of hyperpolarization (Down) and depolarization can be seen more clearly than figure 3A.

The distribution of the filtered signal shows the bimodal characteristics as expected by bistable system. However, there is an overlapping region of these two distributions which makes it difficult to directly distinguish states. Therefore, an expectation maximization algorithm is used to fit this distribution by a mixture of two Gaussians with means μ_{UP} & μ_{DOWN}, and standard deviations σ_{UP} & σ_{DOWN}.

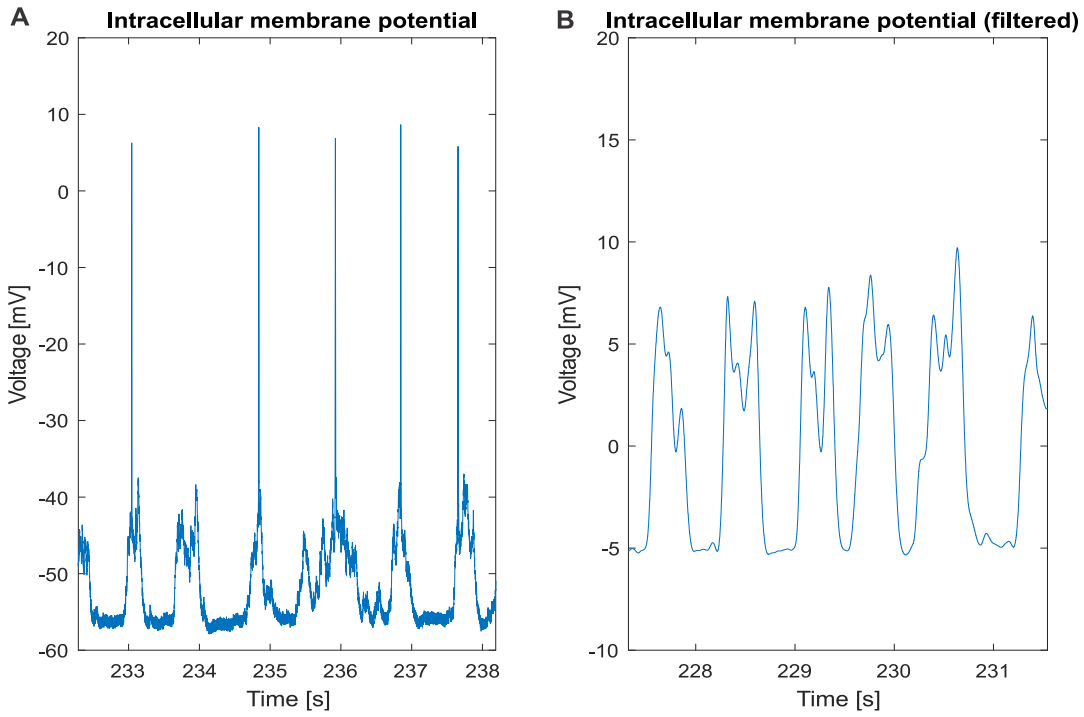

Figure 3 : Processing of whole cell intracellular recording to have only slow alternating hyperpolarized and depolarized (Up and Down) states. A) Intact whole cell intracellular recording with spikes and fast changes in membrane potential. B) Processed whole cell intracellular recording without any spikes and fast abrupt changes in membrane potential, Up and Down states are clearly visible

The upper threshold for UP states is set at $\mu_{UP} - \sigma_{UP}$ and lower threshold to detect DOWN states is set at $\mu_{DOWN} + \sigma_{DOWN}$. We then take only those periods for states where the signal persists above or below the threshold longer than 100 ms. P (UP) is the overall probability

Of an UP state, and is calculated as:

$$P(UP) = \frac{time\ in\ UP\ state}{total\ time}$$

P (DOWN) is also calculated similarly.

1.2 Overview of methods used to detect Up and Down states:

1.2.1 Mukovski method:

After the detection of Up and Down states from membrane potential, authors project the time stamps of the same detected periods of states on simultaneously recorded Local Field Potential (LFP) signal (Mukovski et al., 2007). Thereafter authors calculate the power spectrum of Up and Down state labeled part of LFP signal, separately, using Fast Fourier Transformation (FFT). Upon calculating the ratio of power spectrum of Up and Down state labeled part of LFP signal, there is a clear indication of more power of [10, 200 Hz] band in Up state. But the differences were significantly different mainly in the range 20 to 100 Hz, beta/gamma frequency band.

Because beta/gamma band (20-100 Hz) has preferences for Up state than Down state, Mukovski et al decided to use this difference for state detection without using intracellular recording as ground truth. Firstly, authors convert LFP signal's time domain representation (figure 4A) to frequency domain by performing Fast Fourier Transformation (FFT). Then set in the result (in frequency domain) all coefficients, which corresponded to frequencies below 20 Hz and above 100 Hz to zero. To convert the manipulated frequency domain signal to time domain, they perform Inverse Fast Fourier Transformation (IFFT). After applying these abovementioned steps to LFP signal in figure 4A, the processed signal shows a high correlation to Up and Down states, shown in figure 4B. High fluctuating signal in figure 4B corresponds to Up and small slow fluctuation to Down states. In the second step, authors process the filtered signal with the aim to make the differences between the periods of high amplitude fluctuations and low amplitude fluctuations noticeable. They calculate standard deviation in a running frame of 5 ms, as in figure 4C. The resulting processed LFP signal shows clear correlation with the membrane potential [8]. This similarity increases further after smoothening the obtained trace with a 50 ms running frame linear filter, as in left of figure 4D. Distribution of this processed LFP signal is bimodal, on right of figure 4D.

After obtaining the bimodal distribution from processed LFP signal, authors fit a mixture of two Gaussians to this distribution and then calculate the statistics of fitted Gaussians. Threshold is then calculated from the statistics to detect Up and Down states from processed LFP signal. States detected by this method using LFP can be compared with the ground truth, states detected by whole cell intracellular recording, to quantify how well the states are being detected from LFP. In conclusion, any LFP signal containing Up and Down states can be processed with this method and statistics of bimodal distribution (as shown on right of 4D) of processed signal can be used to detect Up and Down states in original LFP signal.

Steps of LFP processing

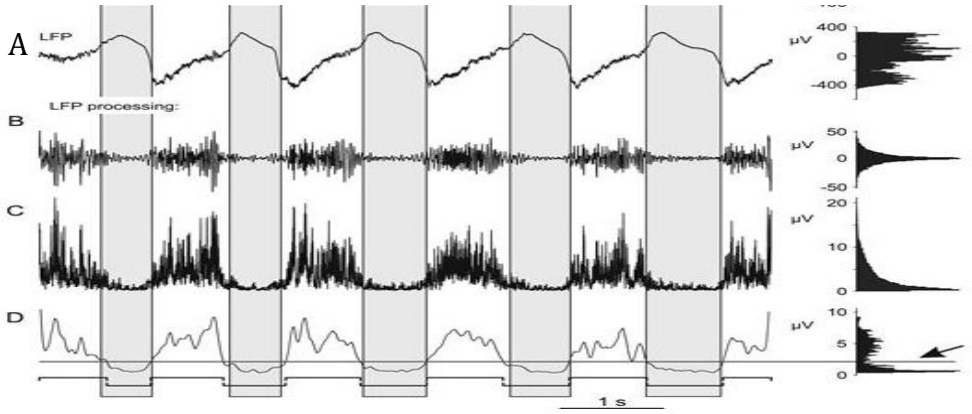

Figure 4 : Steps involved in the processing of LFP for state detection by using the knowledge of differential spectral content [3]. A) Local Field Potential (LFP) with gray boxes as Down states and white area between two gray boxes as Up state and histogram of LFP signal on the right. B) Signal after removing spectral content out of [20, 100] Hz range and its histogram plot on right. C) Signal obtained after applying a standard deviation filter with a 5 ms window on B and on right signal's histogram distribution. D) Signal obtained after smoothening of signal in C, here the histogram distribution shows biomodality which is used for state detection, the line indicated by the arrow in histogram (modified from Mukovski et al, 2007)

1.2.2 Saleem method:

Authors start processing raw LFP signal by filtering out frequencies above 200 Hz using a low pass elliptical filter and then down sampling it from 20 kHz, sampling frequency, to 1000 Hz. The down-sampled LFP signal is then filtered into different frequency bands using second order elliptical low or bandpass filters (peak to peak ripple of 0.1db and minimum stop-band attenuation of 40 dB). In order to avoid phase delays in the filtering process authors use zero-phase filters (Saleem, Chadderton, Apergis-Schoute, Harris, & Schultz, 2010).

The phase $\Phi_X(t)$ and power $k_X(t)$ (where x represents a particular frequency band) at any time instant t in the signal were calculated as the angle and amplitude of the analytic signal (using Hilbert transform) of the LFP signal in each frequency band x. Hilbert transform induces +- 90 degree phase in frequency representation of time series and thereby allowing to have an analytic representation (representation in complex a plane: a + i*Hilbert Transform (a)) of a time series. Analytic representation of a time series allows to calculate instantaneous phase and power of the time series.

Phase of LFP and cortical state:

Given the phase of LFP,$\Phi_X(t)$, from Hilbert transform in a particular frequency band x, we estimate the conditional probability of observing an Up state (based on membrane potential detected state), $P(\mathrm{UP}|\Phi_X(t)$, as

$$P(UP|\Phi_X(t) = \frac{\#((UP|\Phi_X(t),)}{\Phi_X(t)}$$

Where #(x) represents occurrence counts of x. $P(DOWN|\Phi_X(t)$ was also estimated the same way.

We then calculate the differential likelihood of states, $L_x(t)$, as

$$L_x(\Phi_x) = P(UP|\Phi_X(t) - P(DOWN|\Phi_X(t)$$

in different frequency bands. $L_x(\Phi_x)$ ranges between -1 and 1. The relationship between $L_x(\Phi_x)$ and $\Phi_X(t)$, is fitted to a sine wave by optimized $\theta_x(i)$ using mean square error.

Detection of UP and DOWN states from LFP:

The differential likelihood L_x, measures the chance of an Up (L_x being close to 1) or Down (L_x being close to -1). L_x at any time t is approximated by $L_x^{\sim}(t)$ as:

$$L_x^{\sim}(t) = \cos(\Phi_X(t) - \theta'_x(i))$$

Where $\theta'_x(i)$ is averaged value from other whole-cell intracellular recordings.

To detect cortical Up and Down states from LFP, the likelihood from different frequency bands is combined to generate an evidence variable, S_{LFP}. S_{LFP}(t) was used to detect the instantaneous state and was calculated as:

$$S_{LFP}(t) = \frac{1}{2}(1 + \sum K_X(t)L_x^{\sim}(t))$$

Where $K_X(t)$ is the relative power of frequency band x at time t, calculated as

$$K_X(t) = \frac{k_X(t)}{K_{high}(t) + \sum_x k_X(t)}$$

Where $K_{high}(t)$ is the power in the band from 20 to 100 Hz [9].

To determine the threshold for network's instantaneous state, authors fitted the single evidence variable to a mixture of 3 Gaussians using an expectation maximizing algorithm. After fitting, we obtain mean and standard deviation that are μ_{Up-LFP} , $\mu_{IND-LFP,}$ $\mu_{Down-LFP}$ and σ_{Up-LFP} , $\sigma_{IND-LFP}$, $\sigma_{Down-LFP}$, for the Up, Intermediate and Down cortical states, respectively. The thresholds for Up and Down states were selected as $\sigma_{Up-LFP} - n * \sigma_{Up}$ and $\sigma_{Down-LFP} - n * \sigma_{Down}$, respectively. Where n can 1 or 2.

Depending on the experiment's demand and user's choice, evidence variables obtained from two abovementioned methods can be combined.

2. Results:

2.1 Results of state detection from intracellular recording

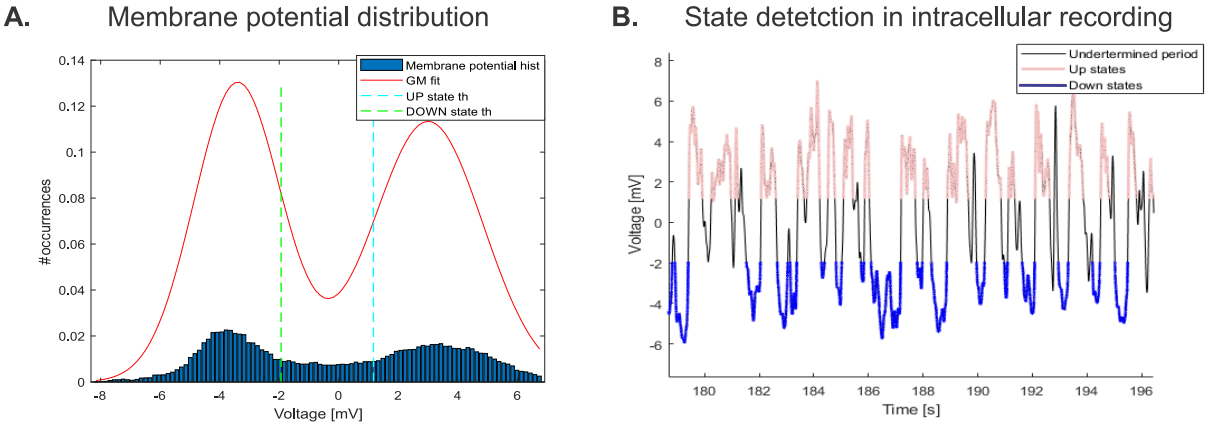

Figure 5: Up and Down state detection from intracellular recording. A) Shows the histogram plot of membrane potential, fitting of a mixture of two Gaussians and thresholds for Up and Down states. B) Shows the Up states (red part of the trace) and Down states (blue part of the trace), obtained by the thresholds shown A

In the methods part, we described the filtering procedure of whole-cell intracellular recordings (example: figure 3A) to have only the slow alternating periods of depolarization (Up) and hyperpolarization as in figure 3B. After plotting the binned membrane potential and fitting the distribution with a mixture of two Gaussians, we estimate the mean and standard deviation of each fitted Gaussian to calculate the Up and Down state threshold (Up state threshold (cyan line in figure 5A): mean1-2*standard deviation1 and Down threshold (green line in figure 5B): mean2-2*standard deviation2). We use the calculated Up and Down thresholds to determine the periods of Up and Down in filtered intracellular recording (figure 5B). In figure 5B pink color labeled period of intracellular recording are Up state periods and blue labeled are periods of Down states. The periods between Up and Down states are considered undetermined periods/transition periods. At this stage of processing, we have the information about periods of Up and Down states from intracellular recording and these periods can be used as ground truth for the detection of network state. These periods of Up and Down state provide the basis for next step processing.

The next steps of the analysis were to really find the properties that can provide a better distinction between Up and Down states in Local Field Potential (LFP) and subsequently estimation of periods of Up and Down states without needing whole cell intracellular recording. With the knowledge of periods of Up and Down states from intracellular recording (figure 5B),

we project these periods on LFP trace as it is shown in figure 6A. In next step, we wanted to know how LFP correlate with the detection of a network state.

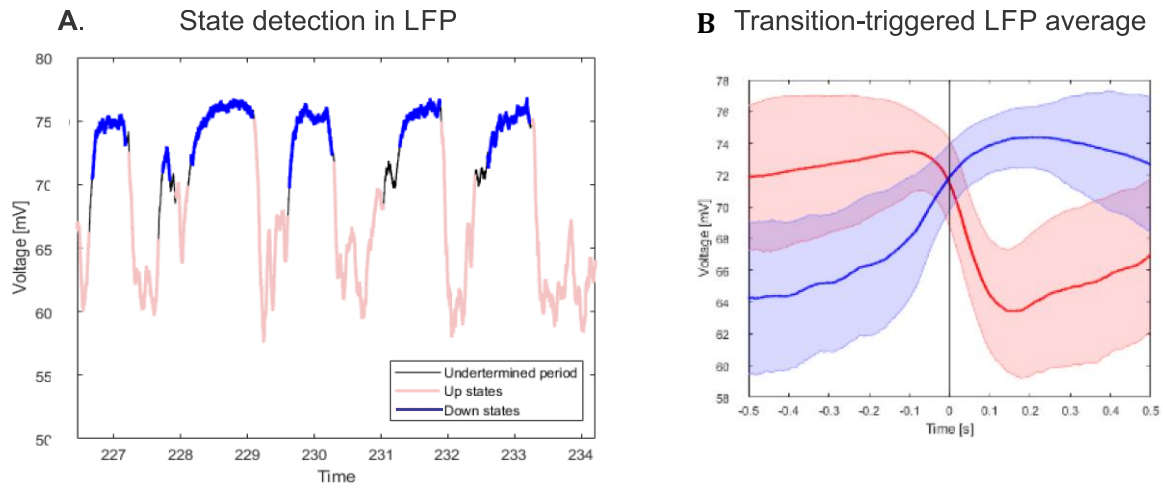

Figure 6: Projecting time stamps of Up and down states obtained from intracellular recording on simultaneously recorded Local Field Potential (LFP) trace and transition-triggered LFP average for Up and Down states

We chose a window of 1000 sample points and calculated the LFP signal change for Up state transition (when LFP detects the Up state) from Down state and Down state transition (when LFP detects the Down state) and then took the average of signal changes around all Up and all Down states separately which is shown in figure 6B (0 on x axis is when the membrane potential detects the Up/Down states). It can be observed in figure 6B that LFP signal starts to go down even before membrane potential detects the Up state and lasts for 150 ms after the detection. Which is consistent with the average time period of Up state detected by membrane potential. We can see the similar fluctuation for Down state but in opposite manner.

In the next step, authors of the both methods thought of examining any possible difference between frequency content that can be basis of detection of Up states without needing whole-cell intracellular recording. We estimate the Power Spectral Density (PSD) of whole LFP signal using Welch's average estimator and for Up state and Down state (detected by intracellular recording) by using averaged FFT. Figure 7A shows the PSD of the entire LFP trace, Up and Down states labeled part of the LFP trace. During Up states, the power of higher frequencies is higher than Down states.

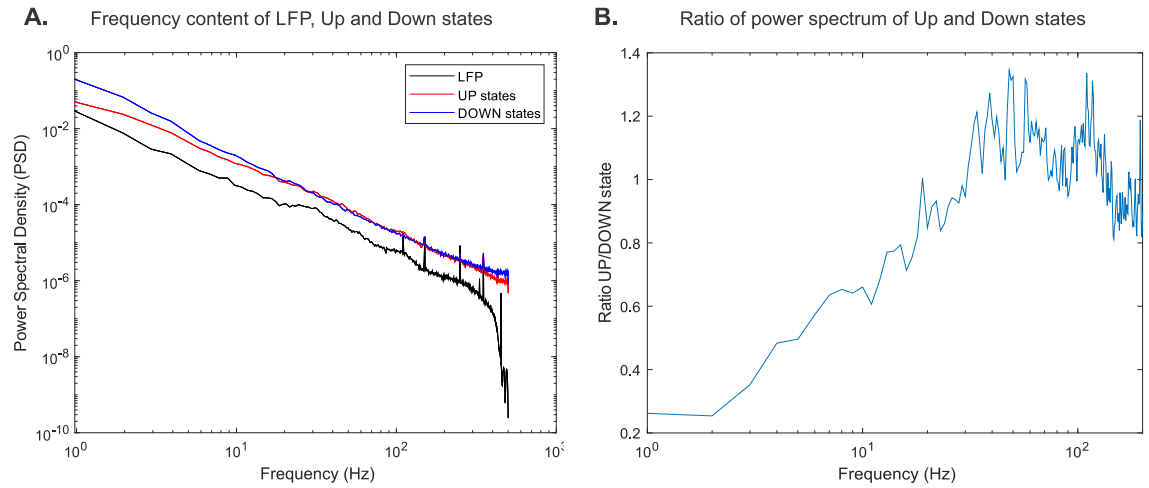

Figure 7: Spectral content of LFP, Up and Down states. A) Plot showing Power Spectrum Density (PSD) of LFP, Up and Down state (averaged). B) Plot showing ratio of power spectrum of Up and Down states

To quantify the PSD differences between Up and Down states, we took the ratio of PSD's of Up and Down states as shown in figure 7B. It can be noticed in the figure 7B that the gamma (20-100 Hz) band contributes significantly to Up state than Down state.

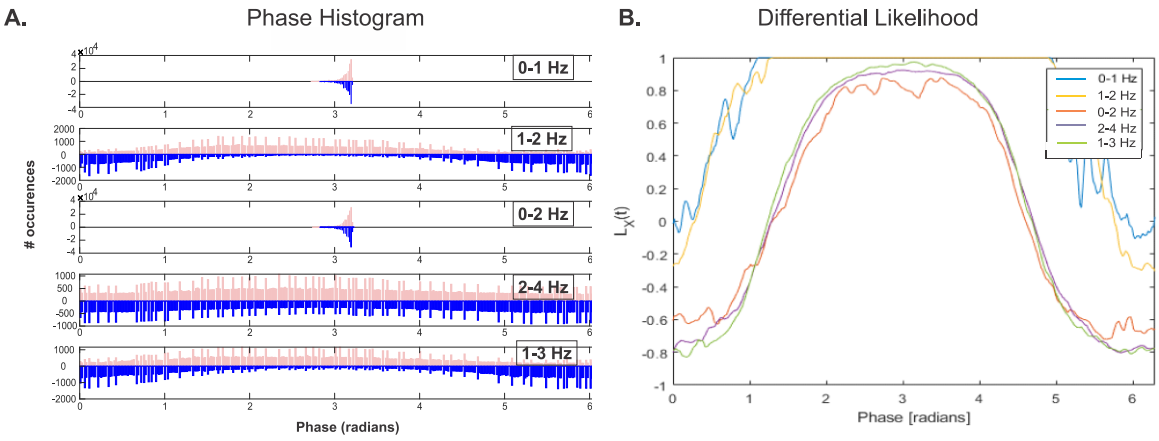

Figure 8: Selective preference of phases for Up and Down states of predefined low frequency bands and differential liklihood for evidence variable. A) Phase histogram of predefined low frequency bands (Pink is preference for Up state and blue for Down states). B) Differential likelihood function $L_x^{\sim}(t)$ fitted to a sine wave for each frequency band

In order to find another feature because gamma band power difference alone doesn't provide a good detection of Up and Down states from LFP, saleem et al examined any possible phase (of a particular bandwidth) preference either for Up or Down state. After calculating instantaneous phase using Hilbert transformation, we plot (figure 8A) the phase of different frequency bands (for our data we choose {0:1, 1:2, 0:2, 2:4, 1:3 in Hz}), mainly bandwidths in lower frequencies, for UP and DOWN states (detected from intracellular recording). In figure 8A, number of

occurrences of a particular phase in Up state are indicated in pink and occurrences of phases in Down states are in green. Essentially, this figure shows conditional probability of Up state given a phase angle phi and same for Down state. In the next step, we calculate the differential likelihood $L_{x(t)}$ (defined by Saleem et al) by subtracting the conditional probability of Down from the conditional probability of UP state. Next, we fit this differential likelihood with a sine wave and calculate the fitting angle θ where L_x gives the value around 1 for each bandwidth. Fitted differential liklihood functions for each frequency band can be seen in figure 8B.

We perform the Mukovski method for 2-3 whole-cell intracellular recordings and simultaneously recorded LFP in order to extract useful bandwidths which can provide better information for the distinction of Up and Down states and angle theta for fitted sine wave. For the evidence variable calculation, we use an average of angle theta obtained from different intracellular recordings for a particular bandwidth.

2.2 Results of state detection from combined evidence variable

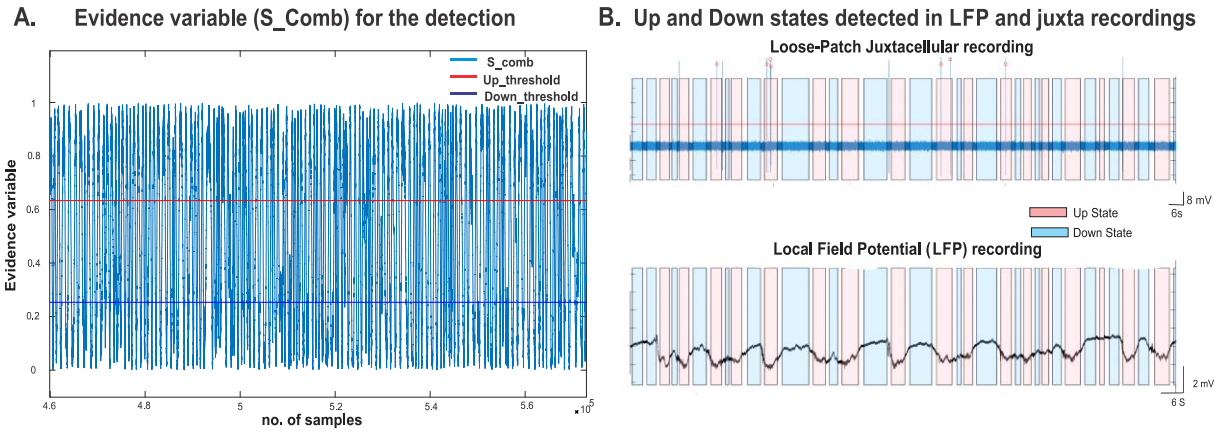

Figure 9: Evidence variable for the determining Up and Down states and detected states in recordings. A) Evidence variable (from single or combined method(s)). B) Periods of Up and Down states plotted on Local Field Potential and loose-patch juxtacellular recording

After obtaining useful bandwidths and theta angles for respective bands by using intracellular recording as ground truth, we calculated our evidence variable (s_comb obtained by combining the resulting evidence variables from both the methods described in methods part) described in methods part and then by fitting a mixture of 3 Gaussians, for Up, intermediate and Down states, to evidence variable (s_comb), we estimate thresholds for Up states and Down states. Figure 9A is a plot of combined evidence variable (s_comb) with the same length as LFP signal. We can see the thresholds for Up states (in red) and Down states (Dark blue) in figure 9A. Up and Down thresholds were estimated from the mean and standard deviation of fitted mixture of Gaussians. We applied the calculated thresholds to s_comb to get the periods of Up and Down as shown in figure 9A (periods below green line, Down state threshold, are Down state periods and periods above red line, Up state threshold, are periods of Up state). After getting period for each detected Up and Down states from the evidence variable (s_comb), we defined some

constraints to reduce the false positives in detection. One of such constraint is the minimum duration of a state which allows selection of only periods having duration greater than the experimenter defined one. We also defined minimum period between two states for our analysis. After applying all the constraints on the detected states, we plot these states on LFP trace as shown in figure 9B. To know the spiking characteristics of single cell during these Up and Down states, we plotted the detected states on simultaneously recorded loose-patch juxtacellular recording, as shown in figure 9B.

3. Discussion:

In past, most strategies for the detection of cortical Up and Down states during anesthesia or NREM sleep relied on detection from whole-cell intracellular recordings (as it shows clear bimodality which allows fitting of a mixture of two Gaussians to the membrane potential to calculate thresholds for Up and Down states). Detection from membrane potential in turn can provide misleading information about the current state of the network because state phenomena is a network phenomenon and detection of this network phenomenon can vary cell to cell because of their intrinsic variability or biasness towards arbitrary fluctuations (Antognini & Carstens, 2002). Here, we show the detection of cortical Up and Down states based on properties (phase of low frequency bands and beta/gamma frequency band) of Local Field Potential that correlate with membrane potential and provide bimodality for the state detection. In our analysis, we used intracellular recording as ground truth of cortical Up and Down state and examined features/properties of LFP in those detected periods of Up and Down state from membrane potential to know if they provide distinction of Up and Down states.

The ratio of power of spectrum of Up and Down states from LFP shows that gamma band significantly contributes to Up state than Down state. More power of gamma band (20-100 Hz) during Up state periods of LFP represents intensive synaptic bombardment and thus high level of activity of network. That's why this feature is ideal to distinguish periods of active (Up) and silent (Down) network activity.

Detection of Up and Down states by using differential gamma frequency band in the states alone is not sufficient to extract permissible/desirable percentage of correct cortical Up and Down states. Therefore, saleem et al explored for other features/properties of LFP that can be provide better detection over differential expression of gamma band. As we know that network is a dynamical system, and that its spontaneous dynamics reflect a continuous trajectory in a high dimensional space. This space can be observed by projecting it onto low dimensional variables. So, authors plotted joint trajectory of LFP and Vm. This trajectory shows that detection of cortical Up and Down states is better when using LFP and Vm than Vm alone. Joint trajectory of low frequencies (<2) and Vm show nontrivial dynamics including hysteresis that suggested that progress can be made in detection by using some properties of low frequencies of LFP. Plotting the phase of low frequencies (<2) suggested that LFP phase may be a good way to look at cortical trajectory and subsequently detect Up and Down states. Based on these observations, saleem et al formulated an evidence variable based on power and phase content of lower frequency bands (Steriade et al., 2011).

In this method, first we define a set of bands in lower frequency (usually <7 Hz) and then see how well they segregate/distinguish in Up and Down state. Based on their segregation, we chose the ones that provides better distinction of Up and Down states. For the calculation of this evidence variable (from saleem et al), it is always beneficial to have several intracellular recordings for robust estimations of bands and angle theta, fitting angle of sine wave fitted differential liklihood function. The distribution of this variable is fitted to a mixture of three Gaussians and from there we can calculate the thresholds for Up and Down states. In our analysis, we are using combination of both of the variables for the detection of Up and Down states.

Main disadvantage of these method/s is having flexibility to choose number of standard deviation (std) to calculate threshold, for example for Up threshold, we use mean + n*std where mean and standard deviation are from one of the fitted Gaussians to evidence variable. Value of n (be it 1 or 2) decides how much undetermined period (longer or shorter) one wants to have while estimating periods of Up and Down. Some user defined parameters can also affect the detection, for example minimum interval between two states, if defined greater the actual interval between two states then the next state will be excluded from detection. Parameters which are crucial and partly based on experimental observations can also sometimes induce subjectivity, therefore user needs to be careful with selection of these parameters while performing analysis based on these evidence variables. The analysis that we present for the detection of cortical Up and Down states is providing with a great extent of accuracy but still it is desirable to have method/s which incorporates another feature of LFP to have a better estimation of Up and Down states.

Conclusions and Perspective:

In the current analysis, we explain Matlab implementation of two methods for the detection of periods of activity (Up) and silence (Down) of a network activity. Each method provides an evidence variable after processing the original LFP signal (processing described in detail in methods) and our implementation combines both evidence variable (s_comb) to detect the Up and Down states.

Mukovski et al observed that gamma frequency band (20-100 Hz) has more power in periods of activity (Up) of LFP when compared with periods of silence (Down) of LFP. This property of LFP was observed with the knowledge of Up and Down state periods from ground truth, whole-cell intracellular recording. By using the differential spectral content of gamma band property of Up and Down states, we processed the LFP signal to obtain an evidence variable that provides the detection of Up and Down states.

Saleem et al method is based on the observation that instantaneous phase of lower frequencies (<7 Hz) of LFP correlate well with membrane potential and provides clear segregation of Up and Down states. Here again this property of LFP was observed with the knowledge of Up and Down states from whole-cell intracellular recording. By using the information about instantaneous phase of lower frequencies of LFP, we formulated an evidence variable that

shows clear bimodality and subsequent fitting of a Gaussian mixture provide the thresholds for Up and Down states.

The response properties of neurons have been shown to change depending on the state of the network. Recent technological advancements are improving our ability to record well isolated spikes trains from a large population of simultaneously recorded neurons using MEA recording (Timofeev et al., 2001), (Steriade et al., 2011). This activity is strongly dependent upon the state of the network under many conditions of interest: to interpret it, it is desired to at the same time have an indicator of the state. Proper detection methods for Up and Down states of a network allow researchers to investigate several cortical processes, network dynamics, synaptic plasticity etc., during these alternating periods of activity and silence. In our lab, we are specifically interested in understanding network dynamics in PFC of Down syndrome and depression (caused by use of Fluoxetine in early age) mouse model during periods of activity and silence induced by ketamine/xylazine.

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Appendices:

Appendix A: Matlab Code

Vikash_intra_UP_DOWN.m

```
% Written by Valentina Pasquel, Tomasso Fellin and Vikash Choudhary
 % vikash_intra_UP_DOWN.m takes whole-cell intracellular and Local Field
 % Potential(LFP) reordings as inputs and returns the useful bandwidths for
state
 % detetction in LFP
   clear all
   close all
   channel = [1 2];
   [dataFilename,pathName] = uigetfile('*.abf','Select your data file'); %
e.g. B100519_0005
   [~,expName] = fileparts(dataFilename);
   [data,si,h] = abf2load([expName '.abf']); %si is the sampling interval
(in us); h is the structure containing all info about the pClamp file
   curSeries_mV = squeeze(data(:,channel(1),:)); %This is the channel
containing the spikes (intracellular or juxta)

   LFPseries_mV = squeeze(data(:,channel(2),:)); %This is the LFP channel
    sf = 1e+6*(1/si);
    pinkColor = [244 194 194]./255;
    medFilterWin = 10e-3;
    bandCutOff = [0.1 20];    % 0.1 Hz has been chosen to cancel drift effects
in the baseline of the membrane potential [0.1 20]
    nBins = 100;
    prctileTh = 99;
    bandCutOffNorm = bandCutOff/(sf);
    [b,a] = butter(2,bandCutOffNorm,'bandpass');
    boundaryWin = 1/bandCutOff(1).*sf; % samples
    % UP & DOWN states detection from membrane potential
    stateDurTh_ms = 100;
    stateIntervTh_ms = 50;
    stateDurTh = stateDurTh_ms*1e-3*sf;
    stateIntervTh = stateIntervTh_ms*1e-3*sf;
    % LFP traces pre-processing
    sfLFP = 1000;    % LFP decimated to 1KHz
    lowPassCutOff = 200; % Hz
    lowPassCutOffNorm = lowPassCutOff/(sf*0.5);
    [bLow,aLow] = ellip(2,0.1,40,lowPassCutOffNorm);
    % PSD computation parameters
    nfft = 128;
    welchWin = 1; %s
    welchWinSamples = welchWin.*sfLFP;
    welchOverlap = round(0.5*welchWinSamples);
    % Transition-triggered analysis of LFP
    halfTransTriggWin = 0.5*sfLFP; % samples
    % Frequency bands for LFP analysis
    fBands = [0 1;1 2;2 3;2 4;1 3];
    phaseMethod = 'hilbert';
    curSeriesMedFilt = medfilt1(curSeries_mV,medFilterWin*sf);
```

```
    %% band-pass [0.1 20] Hz
    %curSeriesLPFilt = filtfilt(b1,a1,curSeriesMedFilt);
    curSeriesLPFilt = filtfilt(b,a,curSeriesMedFilt);
    curSeriesLPFilt = curSeriesLPFilt(boundaryWin+1:end);
    nSamples = length(curSeriesLPFilt);
    %% Plot
    time = (1:length(curSeries_mV)).*1/sf;
    figure(1)
    hold all
    plot(time(boundaryWin+1:end), curSeriesLPFilt,'k');
    xlabel('Time [s]')
    ylabel('Voltage [mV]')
    title('Intracellular membrane potential')
    %% Exclude upper 1 prctile
    outlierTh = prctile(curSeriesLPFilt,prctileTh);
    data2Fit = curSeriesLPFilt(curSeriesLPFilt<=outlierTh);
    figure(2)
    [hAmpl,bins] = hist(data2Fit,nBins);

    bar(bins,hAmpl./length(data2Fit));
    ylabel('# occurrences')
    xlabel('Voltage [mV]')
    hold all
    %% EM fitting by gaussians mixture
    options = statset('Display','final','MaxIter',500);
    try
        GMmodel = gmdistribution.fit(data2Fit,2,'Options',options);   % ill-
posed??
    catch err
        %keyboard;
%         continue; %#ok<BRKCONT>
    end
    mu = GMmodel.mu;
    sigma = squeeze(GMmodel.Sigma);
    figure(2)
    yFit = pdf(GMmodel,bins');
    plot(bins,yFit,'r');
    %% Compute thresholds for UP & DOWN state detection
    [~,idxUP] = max(mu);
    [~,idxDOWN] = min(mu);
    thUP = mu(idxUP)-sqrt(sigma(idxUP));
    thDOWN = mu(idxDOWN)+sqrt(sigma(idxDOWN));
    figure(2)
    line([thUP              thUP],[0              max(yFit)],'LineStyle','--
','LineWidth',1,'Color','c')
    line([thDOWN            thDOWN],[0            max(yFit)],'LineStyle','--
','LineWidth',1,'Color','g')
    legend('Membrane potential hist','GM fit','UP state th','DOWN state th');
    %% Detect UP and DOWN states from intra-cellular trace and save results
    UP_states = curSeriesLPFilt>thUP;
    UP_states_start = find(diff(UP_states)==1);
    UP_states_end = find(diff(UP_states)==-1);
    % correct boundaries
    if UP_states_end(1)<UP_states_start(1)
        UP_states_end = UP_states_end(2:end);
    end
    if UP_states_start(end)>UP_states_end(end)
```

```
        UP_states_start = UP_states_start(1:end-1);
    end
    % join periods of UP-state (above threshold) that are separated by less
    % than a pre-defined time threshold
    UP_states_DET = [UP_states_start UP_states_end]; % samples
    UP_states_interval = UP_states_DET(2:end,1)-UP_states_DET(1:end-1,2);
    UP_states_2join = find(UP_states_interval<=stateIntervTh);
    UP_states_DET(UP_states_2join,2)=UP_states_DET(UP_states_2join+1,2);
    UP_states_DET(UP_states_2join+1,:)=[];
    % delete UP states shorter than a pre-defined time threshold
    UP_state_dur = UP_states_DET(:,2)-UP_states_DET(:,1);
    UP_states_OK = UP_state_dur>=stateDurTh;
    UP_states_DET = UP_states_DET(UP_states_OK,:);
    %
    DOWN_states = curSeriesLPFilt<thDOWN;


    DOWN_states_start = find(diff(DOWN_states)==1);
    DOWN_states_end = find(diff(DOWN_states)==-1);
    if DOWN_states_end(1)<DOWN_states_start(1)
        DOWN_states_end = DOWN_states_end(2:end);
    end
    if DOWN_states_start(end)>DOWN_states_end(end)
        DOWN_states_start = DOWN_states_start(1:end-1);
    end
    % join periods of DOWN-state (below threshold) that are separated by less
    % than a pre-defined time threshold
    DOWN_states_DET = [DOWN_states_start DOWN_states_end]; % samples
    DOWN_states_interval  =  DOWN_states_DET(2:end,1)-DOWN_states_DET(1:end-
1,2);
    DOWN_states_2join = find(DOWN_states_interval<=stateIntervTh);

DOWN_states_DET(DOWN_states_2join,2)=DOWN_states_DET(DOWN_states_2join+1,2)
;
    DOWN_states_DET(DOWN_states_2join+1,:)=[];
    % delete DOWN states shorter than a pre-defined time threshold
    DOWN_state_dur = DOWN_states_DET(:,2)-DOWN_states_DET(:,1);
    DOWN_states_OK = DOWN_state_dur>=stateDurTh;
    DOWN_states_DET = DOWN_states_DET(DOWN_states_OK,:);
    figure(1)
    for uu = 1:length(UP_states_DET)

plot(time(boundaryWin+(UP_states_DET(uu,1):UP_states_DET(uu,2))),curSeriesL
PFilt(UP_states_DET(uu,1):UP_states_DET(uu,2)),'Color',pinkColor,'LineWidth
',2)
    end
    for dd = 1:length(DOWN_states_DET)

plot(time(boundaryWin+(DOWN_states_DET(dd,1):DOWN_states_DET(dd,2))),curSer
iesLPFilt(DOWN_states_DET(dd,1):DOWN_states_DET(dd,2)),'Color','b','LineWid
th',2)
    end
    legend('Undertermined period', 'Up states', 'Down states')
    %% Pre-processing of LFP trace
    % Low-pass filtering of LFP
    LFPseries_mV = LFPseries_mV(boundaryWin+1:end);
    LFPdataFilt = filtfilt(bLow,aLow,LFPseries_mV);
    % Downsampling to 1 KHz
```

```
    nSamplesDec = nSamples/(sf/sfLFP);
    timeDec = (1/sfLFP).*(1:1:(nSamplesDec+1))+boundaryWin/sf;
    LFPdata_dec = decimate(LFPdataFilt,sf/sfLFP);
    % Plot LFP and UP & DOWN states
    UP_states_DET_dec = round(UP_states_DET./(sf/sfLFP));
    DOWN_states_DET_dec = round(DOWN_states_DET./(sf/sfLFP));
    figure(3)
    plot(timeDec', LFPdata_dec,'k');
    hold all
    for uu = 1:length(UP_states_DET)


plot(timeDec(UP_states_DET_dec(uu,1):UP_states_DET_dec(uu,2)),LFPdata_dec(U
P_states_DET_dec(uu,1):UP_states_DET_dec(uu,2)),'Color',pinkColor,'LineWidt
h',2)
    end
    for dd = 1:length(DOWN_states_DET)

plot(timeDec(DOWN_states_DET_dec(dd,1):DOWN_states_DET_dec(dd,2)),LFPdata_d
ec(DOWN_states_DET_dec(dd,1):DOWN_states_DET_dec(dd,2)),'Color','b','LineWi
dth',2)
    end
    title('LFP')
    xlabel('Time')
    ylabel('Voltage [mV]')
    %% Compute and plot PSDs
    [psdLFP,fLFP]                                                    =
pwelch(LFPdata_dec,welchWinSamples,welchOverlap,nfft,sfLFP);
    psdLFP_UP                                                        =
periodogram(LFPdata_dec(UP_states_DET_dec(1,1):UP_states_DET_dec(1,2)),[],n
fft,sfLFP);
    for uu = 2:size(UP_states_DET_dec,1)
        psdLFP_UP                 =                 psdLFP_UP                 +
periodogram(LFPdata_dec(UP_states_DET_dec(uu,1):UP_states_DET_dec(uu,2)),[]
,nfft,sfLFP);
    end
    psdLFP_UP = psdLFP_UP./size(UP_states_DET_dec,1);
    psdLFP_DOWN                                                      =
periodogram(LFPdata_dec(DOWN_states_DET_dec(1,1):DOWN_states_DET_dec(1,2)),
[],nfft,sfLFP);
    for uu = 2:size(DOWN_states_DET_dec,1)
        psdLFP_DOWN               =               psdLFP_DOWN               +
periodogram(LFPdata_dec(DOWN_states_DET_dec(uu,1):DOWN_states_DET_dec(uu,2)
),[],nfft,sfLFP);
    end
    psdLFP_DOWN = psdLFP_DOWN./size(DOWN_states_DET_dec,1);
    %
    figure(4)
    loglog(fLFP,psdLFP,'k');
    hold all
    loglog(fLFP,psdLFP_UP,'r');
    loglog(fLFP,psdLFP_DOWN,'b');
    xlabel('Frequency (Hz)')
    ylabel('Power Spectral Density (PSD)')
    legend('LFP','UP states','DOWN states')
    figure(5)
    semilogx(psdLFP_UP./psdLFP_DOWN)
```

```
    xlabel('Frequency (Hz)')
    xlim([1 200])
    ylabel('Ratio UP/DOWN state')
    %% Compute transition-triggered LFP average
    LFPtriggAvg_UP = zeros(halfTransTriggWin*2,size(UP_states_DET_dec,1));

UP_states_DET_dec(UP_states_DET_dec(:,1)<=halfTransTriggWin|UP_states_DET_d
ec(:,1)>(length(LFPdata_dec)-halfTransTriggWin),:)=[];
    for uu = 1:size(UP_states_DET_dec,1)

    LFPtriggAvg_UP(:,uu)       =       LFPdata_dec(UP_states_DET_dec(uu,1)-
halfTransTriggWin:UP_states_DET_dec(uu,1)+halfTransTriggWin-1);
    end
    LFPtriggAvg_UP_mean = mean(LFPtriggAvg_UP,2);
    LFPtriggAvg_UP_std = std(LFPtriggAvg_UP,[],2);
    %
    LFPtriggAvg_DOWN                                                    =
zeros(halfTransTriggWin*2,size(DOWN_states_DET_dec,1));

DOWN_states_DET_dec(DOWN_states_DET_dec(:,1)<=halfTransTriggWin|DOWN_states
_DET_dec(:,1)>(length(LFPdata_dec)-halfTransTriggWin),:)=[];
    for uu = 1:size(DOWN_states_DET_dec,1)
        LFPtriggAvg_DOWN(:,uu)    =    LFPdata_dec(DOWN_states_DET_dec(uu,1)-
halfTransTriggWin:DOWN_states_DET_dec(uu,1)+halfTransTriggWin-1);
    end
    LFPtriggAvg_DOWN_mean = mean(LFPtriggAvg_DOWN,2);
    LFPtriggAvg_DOWN_std = std(LFPtriggAvg_DOWN,[],2);
    figure(6)
    timeWin = (-halfTransTriggWin:(halfTransTriggWin-1))./sfLFP;

shadedErrorBar(timeWin,LFPtriggAvg_UP_mean,LFPtriggAvg_UP_std,{'LineWidth',
2,'Color','r'},1);
    hold all

shadedErrorBar(timeWin,LFPtriggAvg_DOWN_mean,LFPtriggAvg_DOWN_std,{'LineWid
th',2,'Color','b'},1);
    line([0 0],ylim,'LineWidth',1,'Color','k')
    xlabel('Time [s]')
    ylabel('Voltage [mV]')
%
    %% Filter LFP in different frequency bands
    LFPdataDecFilt = zeros(length(LFPdata_dec),size(fBands,1));
    for bb = 1:size(fBands,1)
        if fBands(bb,1) == 0 % lowpass
            [b_LFPBandFilt,a_LFPBandFilt]                               =
ellip(2,0.1,40,fBands(bb,2)./sfLFP,'low');
        else    % bandpass
            [b_LFPBandFilt,a_LFPBandFilt]                               =
ellip(2,0.1,40,fBands(bb,:)./sfLFP);
        end
        LFPdataDecFilt(:,bb)                                            =
filtfilt(b_LFPBandFilt,a_LFPBandFilt,LFPdata_dec);
    end
    %% For each frequency band, determine instantaneous amplitude and phase
    UP_states_signal_dec = false(size(LFPdata_dec,1),1);
    for uu = 1:length(UP_states_DET_dec)
```

```
UP_states_signal_dec(UP_states_DET_dec(uu,1):UP_states_DET_dec(uu,2))=true;
    end
    DOWN_states_signal_dec = false(size(LFPdata_dec,1),1);
    for uu = 1:length(DOWN_states_DET_dec)

DOWN_states_signal_dec(DOWN_states_DET_dec(uu,1):DOWN_states_DET_dec(uu,2))
=true;
    end
    K = zeros(size(LFPdataDecFilt,1),size(fBands,1));
    phi = zeros(size(LFPdataDecFilt,1),size(fBands,1));
    for bb = 1:size(fBands,1)
        [K(:,bb),phi(:,bb)]                                                    =
UP_DOWN_DET_compInstPhaseAmpl(LFPdataDecFilt(:,bb),phaseMethod);
    end
    phi(phi<=0) = phi(phi<=0)+2*pi; % rescale from 0 to 2*pi
    phi = round(phi./(2*pi/360)).*(2*pi/360);
    %% Plot phase distribution functions during UP & DOWN states in the
different frequency bands
    K_UP = K(UP_states_signal_dec,:);
    K_DOWN = K(DOWN_states_signal_dec,:);
    phi_UP = phi(UP_states_signal_dec,:);
    phi_DOWN = phi(DOWN_states_signal_dec,:);
    figure(7)
    for bb = 1:size(fBands,1)
        subplot(size(fBands,1),1,bb);
        [counts,centers] = hist(phi_UP(:,bb),360);
        bar(centers,counts,'EdgeColor',pinkColor,'FaceColor',pinkColor)
        hold all
        [counts,centers] = hist(phi_DOWN(:,bb),360);
        bar(centers,-counts,'EdgeColor','b','FaceColor','b')
        xlim([0 2*pi]);
    end
    title('Phase histograms')
    %% Differential likelihood of states
    phi_values = unique(phi);
    P_UP = zeros(length(phi_values),size(fBands,1));
    P_DOWN = zeros(length(phi_values),size(fBands,1));
    L_UP_DOWN = zeros(length(phi_values),size(fBands,1));
    L_fit = zeros(length(phi_values),size(fBands,1));
    theta = zeros(1,size(fBands,1));
    legendStrings = cell(1,size(fBands,1));
    for bb = 1:size(fBands,1)
        for ff = 1:length(phi_values)
            P_UP(ff,bb)                                                        =
mean(UP_states_signal_dec(phi(:,bb)==phi_values(ff)));
            P_DOWN(ff,bb)                                                      =
mean(DOWN_states_signal_dec(phi(:,bb)==phi_values(ff)));
        end
        L_UP_DOWN(:,bb) = smooth(P_UP(:,bb)-P_DOWN(:,bb));
        cosFit = fittype('cos(x-theta)');
        fitObj = fit(phi_values,L_UP_DOWN(:,bb),cosFit);
        L_fit(:,bb) = feval(fitObj,phi_values);
        theta(1,bb) = mod(fitObj.theta./(2*pi/360),360);
        legendStrings{bb}              =              [num2str(fBands(bb,1)),'-
',num2str(fBands(bb,2)),' Hz'];
    end
```

```
    figure(8)
    plot(phi_values,L_UP_DOWN/(std(L_UP_DOWN)))
    xlim([0 2*pi])
    legend(legendStrings);
    xlabel('Phase [radians]')
    ylabel('L_X(t)')
   %% Save results


saveas(1,fullfile(pathName,[expName,'_INTRA_DET_Vm_filtTrace.fig']),'fig');
    close(1)

saveas(2,fullfile(pathName,[expName,'_INTRA_DET_Vm_distrib.fig']),'fig');
    close(2)

save(fullfile(pathName,[expName,'_INTRA_DET_results.mat']),'UP_states_DET',
'DOWN_states_DET','boundaryWin');

saveas(3,fullfile(pathName,[expName,'_INTRA_DET_LFP_trace.fig']),'fig');
    close(3)
    saveas(4,fullfile(pathName,[expName,'_INTRA_DET_LFP_PSD.fig']),'fig');
    close(4)

saveas(5,fullfile(pathName,[expName,'_INTRA_DET_LFP_ratioPSD.fig']),'fig');
    close(5)

saveas(6,fullfile(pathName,[expName,'_INTRA_DET_LFP_transTrigg.fig']),'fig'
);
    close(6)

saveas(7,fullfile(pathName,[expName,'_INTRA_DET_LFP_phaseHist.fig']),'fig')
;
    close(7)

saveas(8,fullfile(pathName,[expName,'_INTRA_DET_likelihood.fig']),'fig');
    close(8)
    save(fullfile(pathName,[expName, '_theta_X.txt']),'theta','-ASCII');
```

Spike_detect_In_vivo_UP_DOWN_states.m

```
%Spike_detect_In_vivo_UP_DOWN_states.m takes detected threshold for UP and
%DOWN states, LFP and juxtacellular recordings, minimum period for states
%and between states, bandwidths to use for detection and which methods to
%use
warning off all
clear all
close all
clc

% Load pClamp Files into MATLAB and plot and define important parameters
channel = [1 2];
```

```
threshold = 1.5; %Set the threshold to detect spikes
peakdistance = 50;
[dataFilename,pathName] = uigetfile('*.abf','Select your data file'); % e.g.
B100519_0005
[~,expName] = fileparts(dataFilename);
filename = dataFilename;
```



```
[data,si,h] = abf2load([expName '.abf']); %si is the sampling interval (in
us); h is the structure containing all info about the pClamp file
SPKs = squeeze(data(:,channel(1),:)); %This is the channel containing the
spikes (intracellular or juxta)
LFP = squeeze(data(:,channel(2),:)); %This is the LFP channel
time = (0:si:(length(data)-1)*si)*1e-6; % time in seconds
numchannels = size(channel, 2);
Fs=1/si*1e+6; %Original sampling frequency in Hz
dt = si*1e-6;      % Original sampling interval [s]
Nsamples = size(LFP,1);     % Original length of the waveform, in points
T = Nsamples*dt;       % Original length of the waveform, in s
bandwidth_LFP = input('Please enter the LFP bandwidths from the avalaible
bands (e.g. [0 1; 0 2]): '); %these bands can be extracted from sample
intacellular recordings analyzed with vikash_intra_UP_DOWN.m
minInterv = input ('Please enter the minimum interval between states (in
samples; e.g. 50): ');
minDuration = input ('Please enter the minimum duration of a states (in
samples; e.g. 100): ');

%Highpass filter the Spike signal to remove drift
Fc  = 100;                   % Higher cut-off frequency [Hz]
[bspks,aspks] = butter(8, Fc/(Fs/2.), 'high');
fSPKs = filtfilt(bspks,aspks,double(SPKs)); %do this filtering by the filter
that we just built

% Highpass filter the LFP signal from for MUAs
MUA_H  = 2000;                  % Higher cut-off frequency [Hz]
[MUA_b,MUA_a] = butter(8, MUA_H/(Fs/2.), 'high');
MUA = filtfilt(MUA_b,MUA_a,double(LFP)); %do the highpass filtering
invMUA = -(MUA);


% Let's decimate the LFP signal from Fs to Fs_dec (Fs_dec < Fs) and filter
% the signal below 200 Hz
Fp = 200; % cut off frequency
[bLow,aLow] = ellip(2,0.1,40,Fp/(0.5*Fs));  % A lowpass filter at the cutoff
frequncy 200 Hz
data = filtfilt(bLow,aLow,LFP);
data = data-mean(data);
Fs_dec  = 1000;          % [Hz] New sampling rate
R  = Fs / Fs_dec;  % This is needed for the function decimate()
dLFP = decimate(data, R); % From Fs Hz to Fs_dec Hz
si_dec = (1./Fs_dec);         % Sampling interval, after decimation
dtime = 0:si_dec:(T-si_dec); % new time axis [s]   ...

%This should fix the extra sample bug
if size(dtime,2)>size(dLFP,1)
    dtime = 0:si_dec:(T-si_dec);
    elseif size(dtime,2)<size(dLFP,1)
```

```
    dtime = 0:si_dec:T;
end


%Let's band-pass filter the decimated LFP from LOW to HIGH
LOW   = 20.;                  % Lower cut-off frequency [Hz]
HIGH  = 100.;                 % Higher cut-off frequency [Hz]
[b,a] = butter(8, [LOW/(Fs_dec/2.) HIGH/(Fs_dec/2.)]);
filtered_LFP = filtfilt(b,a,double(dLFP)); %do this filtering by the filter
that we just built

% Detect spikes according to the threshold, and visualize detected spikes
figure(1)
A = subplot(411);
plot(time, fSPKs)
[pks, locs]=findpeaks(fSPKs, 'MinPeakHeight', threshold, 'MinPeakDistance',
peakdistance); %Detect spikes in the intra or juxta channel using findpeaks
peaktimes = locs.*dt; %Times of spikes (in s)
hold on
scatter(peaktimes, pks,'r');%Place a red marlker where each spike is found
plot([0 T], [threshold threshold], 'r') %visualzie the threshold with a red
line
xlabel('Time (s)');
ylabel('mV');

B = subplot(412);
plot(dtime, filtered_LFP, 'k') %Plot the decimated and filtered LFP (in the
20-100 Hz range)
xlabel('Time (s)');
ylabel('Filtered LFP (mV)');

C = subplot(413);
plot(time, LFP, 'r') %Plot the unfiltered LFP
xlabel('Time (s)');
ylabel('LFP (mV)');

D = subplot(414);
% build the spectrogram
movingwin       = [0.5 0.05]; % Size [s] and overlap [s] of the moving window
params.Fs       = 1000;       % Sampling rate [Hz] of the (decimated) LFP
params.fpass    = [10 100];    % Range of frequency to plot [Hz]
params.tapers   = [5 9];      % Multi-taper mysterious parameters
params.trialave = 0;          % Whether (1) or not (0) to perform an average
across all trials
params.err      = 0;         % Whether (1) or not (0) produce the statistical
error in the output

odLFP = dLFP - mean(dLFP);    % Remove offset
[S1,t,f]= mtspecgramc(odLFP, movingwin, params);
plot_matrix(S1,t,f);
colormap jet;
colorbar off;
linkaxes([A, B, C, D], 'x');

figure(2)
```

```
plot_matrix(S1,t,f);
colormap jet;
spikefreq = length(pks)/(time(end));
x = peaktimes(2:end);
ISI = x-peaktimes(1:end-1);
instfreq = 1./ISI;

%display LFP and MUAs
figure(3)
MA = subplot(211);
plot(time, LFP, 'r')
xlabel('time')
ylabel('LFP')
MB = subplot(212);
plot(time, MUA, 'k')
xlabel('time')
ylabel('MUA')
linkaxes([MA, MB], 'x');

%collect all data in a structure
Result_Spikes=struct;
Result_Spikes.Peak = pks;
Result_Spikes.PeakTime = peaktimes;
Result_Spikes.SpikeFreq = spikefreq;
Result_Spikes.InterSpikeInterval = ISI;
Result_Spikes.InstFrequency = instfreq;

save([filename '_preprocessed.mat'],'dt', 'LFP', 'peaktimes');

figure(4)
plot(time, SPKs)
hold on
plot(time, fSPKs)
xlabel('time')
ylabel('SPKs & fSPKS ')

%% Analyze UP and DOWN states (from Valentina Pasquale and Tommaso Fellin)
sf = Fs_dec;
selectedBands = bandwidth_LFP;
phaseMethod = 'hilbert';
bands = {'1-2','2-4','3-5','1-4'};
theta = [3.1048,3.0931];
nSTD_UP = 2;
nSTD_DOWN = 2;
fHigh = [20, 100];
combFlag = 0;
rmsWin = 5;
smoothWin = 51;
nfft = 1024;
welchWin = 2; %s
welchWinSamples = welchWin.*sf;
welchOverlap = round(0.5*welchWinSamples);
nsamples_dec = length(dLFP);
```

```
 [S_comb,S_LFP,S_2,mu,sigma,thUP,thDOWN,GMmodel]                                    =
estimateUPDOWNdetTh(dLFP,sf,selectedBands,phaseMethod,bands,theta,nSTD_UP,n
STD_DOWN,fHigh,combFlag,rmsWin,smoothWin); %Set the threshold for UP and DOWN
state detection

[UP_states_DET,  DOWN_states_DET]  =  UP_DOWN_DET_detectStates(S_LFP,  thUP,
thDOWN, minInterv, minDuration); %Detect UP and DOWN states

figure(5)
plot(S_comb)
xlabel('time')
ylabel('S comb (evidence variable)')
legend('S_comb','thUP','thDOWN')
hold on
plot([0 size(S_comb,1)], [thDOWN thDOWN], 'b')
plot([0 size(S_comb,1)], [thUP thUP], 'r')
%% LFP with Up and Down states
%Plot the decimated LFP and mark the beginning and end of each UP and DOWN
state.
%Red and green horizontal bars are UP and DOWN states, respectively
nsamples = nsamples_dec*(Fs/Fs_dec);
% Plot LFP and UP & DOWN states
UP_states_DET_org = round(UP_states_DET.*(Fs/Fs_dec));
DOWN_states_DET_org = round(DOWN_states_DET.*(Fs/Fs_dec));
time_org = 1/Fs.*(1:1:nsamples);
figure(6)
plot(data, 'k')
xlabel('time')
ylabel('LFP (orignal)')
hold on

for iUP = 1: size(UP_states_DET_org, 1)
    x1 = UP_states_DET_org(iUP,1);
    x2 = UP_states_DET_org(iUP,2);
    x = [x1, x2];
    y1  =  line([x1  x1],[min(data)-0.5  max(data)],'Color','red','LineWidth',
1);
    y2 = line([x2 x2],[min(data)-0.5 max(data)],'Color','r', 'LineWidth', 1);
    patch([x1 x2 x2 x1], [min(data)-0.5  min(data)-0.5 max(data) max(data)],
[255/255,182/255,193/255],'FaceAlpha',0.3)
end


for iDOWN = 1: size(DOWN_states_DET_org, 1)
    x1 = DOWN_states_DET_org(iDOWN,1);
    x2 = DOWN_states_DET_org(iDOWN,2);
    x = [x1, x2];
    y1 = line([x1 x1],[min(data)-0.5 max(data)],'Color','b','LineWidth', 1);
    y2 = line([x2 x2],[min(data)-0.5 max(data)],'Color','b', 'LineWidth', 1);

    patch([x1 x2 x2 x1], [min(data)-0.5  min(data)-0.5 max(data) max(data)],
[130/255 200/255 250/255],'FaceAlpha',0.3)
end


%% Here we can see the distribution of UP state durations
```

```
UP_state_duration = UP_states_DET(:,2)-UP_states_DET(:,1);
figure(7)
hist(UP_state_duration)
xlabel('UP state duration (ms)');

%% Compute and plot PSDs
[psdLFP,fLFP] = pwelch(dLFP,welchWinSamples,welchOverlap,nfft,sf);
psdLFP_UP                                                                =
periodogram(dLFP(UP_states_DET(1,1):UP_states_DET(1,2)),[],nfft,sf);
for uu = 2:size(UP_states_DET,1)
    psdLFP_UP                    =                    psdLFP_UP                    +
periodogram(dLFP(UP_states_DET(uu,1):UP_states_DET(uu,2)),[],nfft,sf);
end
psdLFP_UP = psdLFP_UP./size(UP_states_DET,1);
psdLFP_DOWN                                                              =
periodogram(dLFP(DOWN_states_DET(1,1):DOWN_states_DET(1,2)),[],nfft,sf);
for uu = 2:size(DOWN_states_DET,1)
    psdLFP_DOWN                  =                  psdLFP_DOWN                  +
periodogram(dLFP(DOWN_states_DET(uu,1):DOWN_states_DET(uu,2)),[],nfft,sf);
end
psdLFP_DOWN = psdLFP_DOWN./size(DOWN_states_DET,1);
%
figure(8)
loglog(fLFP,psdLFP,'k');
hold all
loglog(fLFP,psdLFP_UP,'r');
loglog(fLFP,psdLFP_DOWN,'b');
xlabel('Frequency')
ylabel('PSD')
legend('LFP','UP states','DOWN states')
figure(9)
semilogx(psdLFP_UP./psdLFP_DOWN)
xlabel('Frequency')
xlim([1 200])
ylabel('Ratio UP/DOWN')

%% Detect and quantify spikes in each UP states
UP_states_DET_sec = UP_states_DET_org.*(si*1e-6);
DOWN_states_DET_sec = DOWN_states_DET_org.*(si*1e-6);
Spikes_UP_states = struct;
figure(10)
UPa = subplot(211);
plot(time,fSPKs)
for iUP = 1: size(UP_states_DET_org, 1)
    x1 = UP_states_DET_sec(iUP,1);
    x2 = UP_states_DET_sec(iUP,2);
    x = [x1, x2];

    y1 = line([x1 x1],[min(fSPKs) max(fSPKs)-2],'Color','red','LineWidth',
1);
    y2 = line([x2 x2],[min(fSPKs) max(fSPKs)-2],'Color','r', 'LineWidth', 1);
    patch([x1 x2 x2 x1], [min(fSPKs) min(fSPKs) max(fSPKs)-2 max(fSPKs)-2],
[255/255,182/255,193/255],'FaceAlpha',0.3)
end

for iDOWN = 1: size(DOWN_states_DET_sec, 1)
```

```
    x1 = DOWN_states_DET_sec(iDOWN,1);
    x2 = DOWN_states_DET_sec(iDOWN,2);
    x = [x1, x2];
    y1 = line([x1 x1],[min(fSPKs) max(fSPKs)-2],'Color','b','LineWidth', 1);
    y2 = line([x2 x2],[min(fSPKs) max(fSPKs)-2],'Color','b', 'LineWidth', 1);
    patch([x1 x2 x2 x1], [min(fSPKs)  min(fSPKs) max(fSPKs)-2 max(fSPKs)-2],
[130/255 200/255 250/255],'FaceAlpha',0.3)
end
for iUP=1: size(UP_states_DET, 1)
    peaktimes_UP    =    peaktimes(peaktimes>UP_states_DET_sec(iUP,1)    &
peaktimes<UP_states_DET_sec(iUP,2)); %get the spike times (detected above)
within each UP state
    pks_UP = pks(find(peaktimes_UP)); %get the peak of the each spike within
each UP state (used to place a marker in the figure)

    hold on
    scatter(peaktimes_UP, pks_UP,'r');%Place a red marlker where each spike
is found
    plot([0 T], [threshold threshold], 'r') %visualzie the threshold with a
red line
    xlabel('Time (s)');
    ylabel('fSPKS (mV)');
    Spikes_UP_states(iUP).PeakTimes_UP_states = peaktimes_UP;
end

UPb = subplot(212);
plot(time, LFP, 'k')
for iUP = 1: size(UP_states_DET_org, 1)
    x1 = UP_states_DET_sec(iUP,1);
    x2 = UP_states_DET_sec(iUP,2);
    x = [x1, x2];
    y1 = line([x1 x1],[min(LFP)-0.5 max(LFP)],'Color','red','LineWidth', 1);
    y2 = line([x2 x2],[min(LFP)-0.5 max(LFP)],'Color','r', 'LineWidth', 1);
    patch([x1  x2  x2  x1],  [min(LFP)-0.5   min(LFP)-0.5  max(LFP)  max(LFP)],
[255/255,182/255,193/255],'FaceAlpha',0.3)
end


for iDOWN = 1: size(DOWN_states_DET_sec, 1)
    x1 = DOWN_states_DET_sec(iDOWN,1);
    x2 = DOWN_states_DET_sec(iDOWN,2);
    x = [x1, x2];

    y1 = line([x1 x1],[min(LFP)-0.5 max(LFP)],'Color','b','LineWidth', 1);
    y2 = line([x2 x2],[min(LFP)-0.5 max(LFP)],'Color','b', 'LineWidth', 1);
    patch([x1  x2  x2  x1],  [min(LFP)-0.5   min(LFP)-0.5  max(LFP)  max(LFP)],
[130/255 200/255 250/255],'FaceAlpha',0.3)
end
xlabel('Time (s)');
ylabel('LFP (mV)');
linkaxes([UPa, UPb], 'x');
%% Back calculation of spike numbers from detected UP states
%Calculate the number of spikes in each UP state
for i = 1:size(Spikes_UP_states, 2)
Nspks_per_UPstate (i) = numel(Spikes_UP_states(i).PeakTimes_UP_states);
end
```

```
Nspks_per_UPstate = Nspks_per_UPstate'; %output of the number of spikes per
UP State
edges = [0:1:max(Nspks_per_UPstate)];
figure(11)
subplot(121)
hist(Nspks_per_UPstate, edges)
xlabel('Number of spikes per UP state');
ylabel('count');
subplot(122)
avgNumSpikesUPstate = mean(Nspks_per_UPstate); %average number of spikes per
UP state
stdevSpikesUPstate = std(Nspks_per_UPstate); %standard deviation of the mean
number of spikes per UP state
bar(avgNumSpikesUPstate)
hold on
errorbar(avgNumSpikesUPstate, stdevSpikesUPstate)

%% phase analysis
arg = input("Do you want to continue the monte carlo analysis?\n yes\n no\n",
's');
switch arg
    case 'yes'
        disp("Continuing the execution of the script :-) ")
    case 'no'
        return
end
postprocessing_ppc_and_phaseHistogram_2;
save([filename '_Spikes_Phase.mat'],'Result_Spikes', '-append');
```