Cross-trial correlation analysis of evoked potentials reveals arousal-related attenuation of thalamo-cortical coupling

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Abstract:

We describe a computational method for assessing functional connectivity in sensory neuronal networks. The method, which we term *cross-trial correlation*, can be applied to signals representing local field potentials (LFPs) evoked by individual sensory stimulations and utilizes their trial-to-trial variability. A set of single trial samples of a given post-stimulus latency from consecutive evoked potentials (EPs) recorded at a given site is correlated with such sets for all other latencies and recording sites. The results of this computation reveal how neuronal activities at various sites and latencies correspond to activation of other sites at other latencies. The method was used to investigate the functional connectivity of thalamo-cortical network of somatosensory system in behaving rats at two levels of activation: habituated and aroused. We analyzed potentials evoked by vibrissal deflections recorded simultaneously from the ventrobasal thalamus and barrel cortex. The cross-trial correlation analysis applied to the early post-stimulus period (<25 ms) showed that the magnitude of the population spike recorded in the thalamus at 5 ms post-stimulus correlated with the cortical activation at 6-13 ms post-stimulus. This correlation value was reduced at 6-9 ms, i.e. at early postsynaptic cortical response, with increased level of the animals' arousal. Similarly, the aroused state diminished positive thalamo-cortical correlation for subsequent early EP waves, whereas the efficacy of an indirect cortico-fugal inhibition (over 15 ms) did not change significantly. Thus we were able to characterize the state related changes of functional connections within the thalamo-cortical network of behaving animals.

Keywords: *LFP; awake rat; vibrissae-barrel system; functional connectivity*

Introduction

Connections between brain structures can be revealed anatomically (by exposing interconnecting neuronal processes) or by recording the responses evoked in one structure by micro-stimulation at the other location. However, to understand how these connections function and how these structures cooperate, one has to monitor their activity in behaving animals when they are engaged in specific tasks. Development and miniaturization of electrophysiological equipment makes it possible to simultaneously record extracellular signals from multiple locations and thus analyze the dynamics of complicated neural networks. Prolonged chronic recording of neuronal activity at multiple brain sites is best accomplished with local field potential (LFP) technique, as unitary recordings are not sufficiently stable, while EEG is not sufficiently accurate and precludes subcortical recording. However, studies relating brain structure to function can be possible only with corresponding development of appropriate analytical tools, without which the data accessible with multichannel recordings would not be exploited to their full potential.

A number of different methods have been used to extract information about connectivity, connection strength, etc., from electrophysiological signals. Some approaches were based on spike train analysis (such as cross-correlation histograms (Perkel et al. 1967), joint peri-stimulus time histogram (Aertsen et al., 1989), and gravity analysis (Gerstein and Aertsen 1985, Gerstein et al. 1985), other methods utilized LFP; these include: spectral analysis (Wróbel et al. 2007), phase synchronization inferred through wavelet or Hilbert transforms (Lachaux et al. 1999; Rosenblum et al. 1996), nonlinear interdependence (Arnhold et al. 1999; Łęski and Wójcik 2008), a variety of methods based on multivariate autoregressive models, such as coherence or directed transfer function (Ding et al. 2000; Korzeniewska et al. 2008), and methods based on information theory (Schreiber 2000). A comparison of several of these methods can be found in a paper by Quian Quiroga et al. (2002).

Here we propose a simple and effective approach we term *cross-trial correlation*. On a trial-by-trial basis, LFP activity evoked by uniform sensory stimulation (evoked potentials, EPs) is very variable. This variability and its underlying factors have been investigated before (Arieli et al. 1996), but are not fully understood and probably cannot be comprehensively accounted for. The traditional method for dealing with such variability is averaging over many trials, however the importance of studying this variability has also been appreciated (Kisley and Gerstein 1999). The idea of our method is to take advantage of this variability, i.e. to correlate the single-trial values of the repetitive signals (like EPs generated by repeated uniform stimulation) recorded in two locations. However, we do not correlate the time-courses of the signals (in a broad sense this concept underlies most of the methods referred to above). Rather, we analyze how the trial-to-trial variability of one of the signals at a specific time point (specific post-stimulus latency) is reflected in the trial-to-trial variability of the other signal at another time point. In this way, we are able to assess how strongly one signal influences the other and at the same time we do not need to assume that the temporal scales (frequency) of the activity at the two places are the same.

We present the application of the above analysis method to EPs obtained from the ventrobasal thalamus and primary somatosensory cortex (the barrel cortex) of awake rats. The circuitry of this interconnected thalamo-cortical system is relatively well known (Waite 2004). Also the basic rules governing this system's functional reorganization between different processing states have been researched - mainly in anaesthetized rats (e.g. see Aguilar and Castro-Alamancos 2005; Castro-Alamancos and Oldford 2002, on arousal-like state induced by the

brainstem stimulation) or in slice preparations (e.g. see Rigas and Castro-Alamancos 2009 on cortical UP and DOWN states). The state-related changes in thalamic and cortical processing of sensory information have also been investigated in non-anaesthetized animals, however the majority of those studies concerned the sleep-wake cycle (Steriade 1997; Steriade and Timofeev 2003). Fewer studies have addressed shifts in thalamo-cortical transfer of sensory information in behaving, awake animals – when subtle changes of arousal levels (alertness, vigilance) are elicited by demands of the behavioral context (Wróbel et al. 1998; Castro-Alamancos 2004a; Stoelzel et al. 2009). Therefore, these dynamics were the target of our research, and the object of the present paper was to analyze the changes of thalamo-cortical functional connectivity within the rat barrel system in different awake behavioral states.

Methods

Experimental procedures

Seven male Wistar rats weighing 300-400 g were used in the chronic experimental paradigm employed in our laboratory (Kublik 2004). All experimental procedures followed the 86/609/EEC directive and were accepted by the 1st Warsaw Local Ethics Committee. Initially, for ~2-3 weeks, the animals were handled and accustomed to a restraining hammock in the experimental room. Then, under deep chloral hydrate anesthesia (3.6% solution, 10 ml/kg b.w.), surgery was performed during which 1 to 6 electrodes (of 0.025 mm diameter insulated tungsten wire, each tip conically sharpened, ~150 kΩ impedance at 1 kHz) were implanted into the rat's barrel cortex, and 6 to 15 such electrodes into various dorsal thalamic nuclei, contralaterally to the planned whisker stimulations. Several small (ø 1-1.4 mm) stainless steel anchoring screws were inserted into the skull bone; the screws also served as recording reference. The screws, electrodes, connectors and, additionally, a head restraining bolt were secured on the skull with dental acrylic. After the surgery, and during the first few days of recovery, the rats received analgesic drugs (subcutaneous 0.1 ml injections of Rimadyl, or Paracetamol 500 mg per 100 ml of drinking water) and antibiotic (Baytril 0.1 ml sc/24 hr, 5 days).

After the recovery period, the animals were subjected to 4 to 8 daily recording sessions (one hour per day) during which they were habituated to the control conditions of the experiment, i.e. they were suspended in the hammock, head immobilized with the aid of the restraining bolt, and their whiskers stimulated (100 to 120 times during a session, with a pseudo-random inter-trial interval of 10 to 45 s). The whisker stimulations consisted of a 1 ms, ~0.1 mm down-and-up movement of a piezoelectric slab glued to a group of ~20 biggest facial whiskers about 15-20 mm from the snout. After habituation sessions, 1 to 3 experimental sessions followed which consisted of two parts: (i) they started with conditions identical to the habituation sessions, but (ii) after the first 50 to 60 whisker stimulations all the remaining whisker stimulations were intermingled with arousing stimuli: an electric current (0.03 – 0.07 mA, 3 ms square pulse, 1 s train, 50 Hz) applied onto the skin of rat's ear, or (depending on a session) a 1 s ~7000 Hz loud (90 dB) sound from a speaker placed 10 cm from the rat's ear. We refer to the first parts of the experimental sessions as the *control* conditions, and the parts, during which the additional electric/auditory stimuli were applied, as the *arousal* conditions.The procedure (4 to 8 habituations followed by 1 to 3 experimental sessions) was repeated 2 to 5 times (depending on the rat) for each rat.

Throughout all sessions LFP signals from the implanted electrodes were recorded (with a 0.1 – 5000 Hz band pass filter, amplification x 1000) to a data file (Spike 2 software, version

5.15, Cambridge Electronic Design, UK) after on-line digitalization at 10 kHz sampling frequency with an analog-digital interface (CED 1401, Cambridge Electronic Design, UK), or to a VHS tape recorder (RACAL V-store, Racal Recorders, UK) and then digitized off-line. After completion of the recordings, the rats were injected intra-peritoneally with an overdose of pentobarbital (150 mg/kg b.w.), perfused transcardially with saline followed by 4% paraformaldehyde. Brains were removed, cryoprotected in 30% sucrose, cut into 50 μm slices and stained with cresyl violet and for cytochrome oxidase (alternating from slice to slice) for histological verification of electrode placement.

Preparation of data

Initially, signal sweeps of 1400 ms (400 ms pre- and 1000 ms post-stimulus) containing EPs were extracted from the continuous recordings and exported to Matlab. Since the rats were well habituated to the experimental situation, we observed many episodes of high amplitude oscillations in an approximate frequency range of 5-12 Hz, typical for recordings from the immobile animals (Buzsáki 2006). Such oscillatory activity represents a specific, idle state of the thalamocortical network (possibly sustaining a sensory hypersensitivity optimized for detecting weak sensory signals) reported elsewhere (Fontanini and Katz 2005; Fanselow et al. 2001; Sobolewski, Świejkowski, Wróbel, Kublik, unpublished), and for the present analysis we selected for each rat 100 control and arousal conditions EPs which were not directly preceded by oscillatory events. For further analysis, we used epochs of the first 25 ms post-stimulus, containing the earliest waves of the EPs. To avoid the possible jitter in the amplitudes of EPs from different day-sessions, we normalized the data from each day to a standard score (from every sample we subtracted a mean of all samples and divided it by the standard deviation of all samples). To compensate for any low-frequency fluctuations in the LFP, not brought on by the stimulus, from every epoch we subtracted a baseline value, being the potential measured at the time of the stimulus presentation.

The cross-trial correlation method

Let us assume that we record k evoked potentials at two locations, X and Y. Let us denote the resulting signals by $x_i(t)$ and $y_i(t)$, with i numbering the consecutive trials. Panels (a) and (b) in Fig. 1 present an example of such (artificial) data (only 5 traces out of 100 generated are shown). For any chosen time points t_1 and t_2 we have a collection of measured values (voltages) at the first ($x_i(t_1)$, $1 \le i \le k$) and at the second location ($y_i(t_2)$, $1 \le i \le k$). We calculate the correlation coefficient between these two sets:

$$R(t_1, t_2) = C(x_i(t_1), y_i(t_2)) \, [C(x_i(t_1), x_i(t_1)) \, C(y_i(t_2), y_i(t_2))]^{-1/2},$$

where C denotes covariance: $C(f_i, g_i) = E[(f_i - E(f_i)) \, (g_i - E(g_i))]$, E is the mean. In this way, we obtain a number which quantifies the relation between the LFP value in X at time t_1 and the LFP value in Y at time t_2. We can repeat this procedure for all possible pairs (t_1, t_2), thus obtaining what we call *the correlation matrix R*. Subsequently, we substitute with zeros all elements of this matrix (correlation coefficients) for which the calculated correlation coefficient is not significantly ($p<0.05$) different from zero. The matrix can be conveniently plotted as a color-scale bitmap (Fig. 1(c)), hence we will also refer to any individual element of R as a *correlation matrix pixel*; we will also refer to distinct grouping of such correlation matrix pixels of the same sign as a *cluster*. Mean traces from signal X and signal Y are also added below and on the left of the matrix, respectively, for orientation and viewing convenience, serving to easily relate correlation clusters to corresponding LFP waves (or

latencies). A Matlab code for calculating and viewing the cross-trial correlation is available online at: http://www.nencki.gov.pl/vslab/worek/ctc.html.

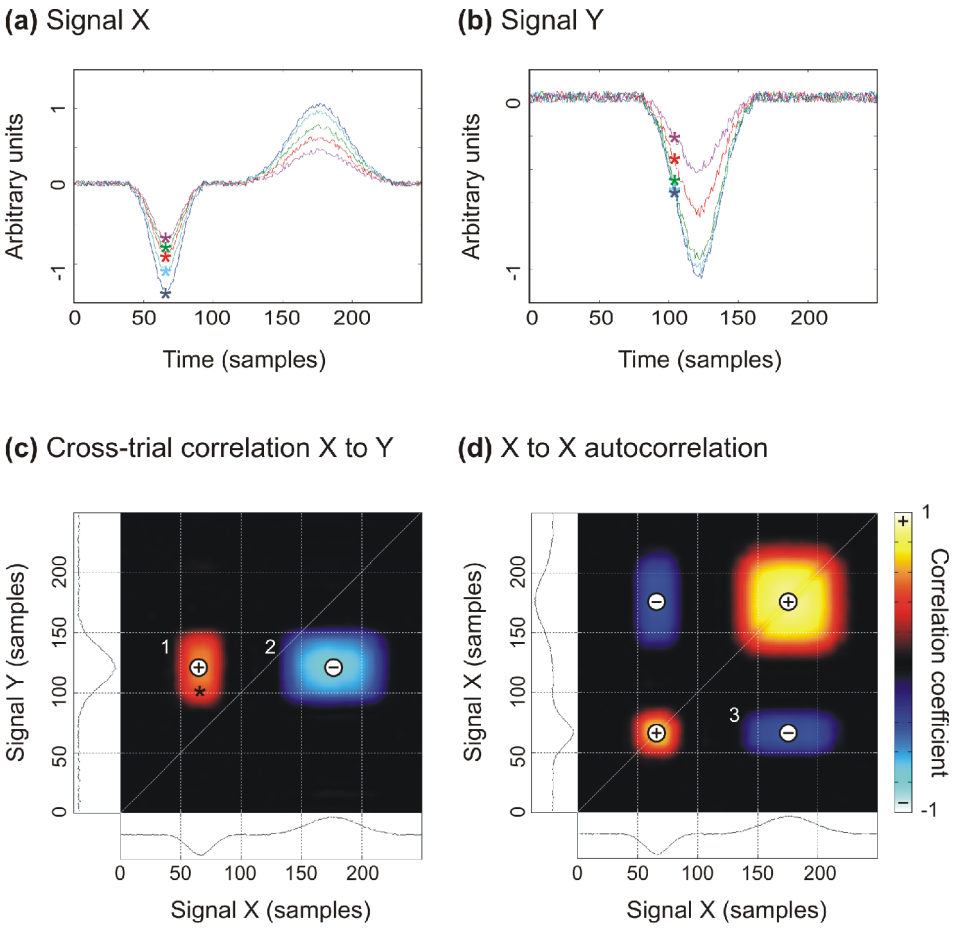

Fig. 1. Example of application of the of cross-trial correlation on an artificial dataset (epochs of 250 samples). The first signal (X) of the artificial data, shown in panel (a), includes a Gaussian profile of varying amplitude at 40th to 90th sample. The second signal (Y) shown in panel (b), is generated by multiplying this profile from X by a random number between 0.9 and 1.1, delaying it by 100 samples, stretching it in time by 1.5. Similarly, the second wave in signal X at 125th to 225th sample, illustrating a feedback connection from location Y, is obtained by the same procedure (multiplying, delaying, stretching) applied to the profile from Y, which is then subtracted from X. Panel (c) shows cross-trial correlation matrix for the two signals. (The matrices were smoothed with a 2-dimensional Gaussian kernel of radius of 15 samples). For orientation and viewing convenience mean traces of signal X and signal Y are added below and on the left of the matrix, respectively. The asterisk in panel (c) indicates the correlation coefficient between the values recorded in the test data at the corresponding latency in channel X (asterisks in panel (a)) and in channel Y (asterisks in panel (b)). Panel (d) shows a similar matrix for auto-correlation of signal X to itself.

The idea behind the measure proposed above is the following: consider, for example, a situation when the two recording sites X and Y are located in brain structures mutually connected, with the neuronal population at X providing excitatory input to Y, and Y providing inhibitory feedback to X. A stronger LFP negativity in X means stronger excitation (e.g. larger amplitude of compound postsynaptic potentials or a greater number of excited neurons), which may cause more cells to fire action potentials. This in turn will translate to a

stronger postsynaptic signal in Y (a negative wave of greater amplitude) after a delay corresponding to the time-scale of axonal and synaptic transmission. In such case the correlation between signal strength in X and Y will be positive, i.e. we would expect a cluster of positive correlation pixels above the diagonal of the correlation matrix (Figure 1(c), cluster 1). On the other hand, if the structures were not connected, the variability of the signal at X would have no effect on the signal at Y, hence the correlation would be close to zero. Similarly, the feedback connection from Y to X will result in a non-zero correlation cluster below the diagonal, i.e. for $t_1 \geq t_2$ (Fig. 1(c), cluster 2). Note that in Fig. 1(d), showing results for autocorrelation of signal X to itself, we can see a cluster of positive correlation (labeled 3) suggesting that the second wave in X is influenced by the first wave from this signal. However, the values of the correlations in cluster 3 are smaller than in cluster 2, suggesting that this influence is indirect (through Y).

To study how the connection strength differs in various experimental situations, we can look at the changes in the correlation matrices *R* obtained in those situations. Therefore the cross-trial correlation method allows to both: hypothesize about the directions of functional influences and their time courses, as well as quantify the strength of the inter-structure coupling.

Application of the correlation method to the experimental data

We applied the cross-trial correlation analysis separately to the control and arousal sets of the normalized 100 EPs in each rat. All available thalamic electrodes (6 to 15 depending on a rat) were paired with a single cortical electrode implanted in infragranular layers (this cortical position was chosen because it encompasses the origin of feedback projections to the thalamus). For each such pair we obtained an individual correlation matrix, as described in the preceding section. Subsequently, for each rat, from all the individual correlation matrices we selected one thalamic site with a signal which yielded the greatest cortical correlation in earliest latencies, i.e. had the highest sum of correlation coefficients (pixels) for 4 to 6 ms latencies in the thalamus and 6 to 13 ms in the cortex. We assumed this site as having the largest afferent impact on the cortical recording and representing the most closely matched barreloid-barrel electrode pair. (Other recording sites and differences between specific thalamic nuclei will be a matter of further analysis.) Corresponding matrices were selected from the data for arousal conditions. This gave us 14 identically formatted matrices, illustrating correlations between the ventrobasal thalamic evoked potentials and infragranular cortical activity – 7 for control, and 7 for arousal conditions. We then calculated two group-averaged correlation matrices (control and aroused) and tested if resulting mean coefficients in each matrix pixel were significantly different from zero (t-test, $p<0.05$) and from each other (paired t-test, $p <0.05$).

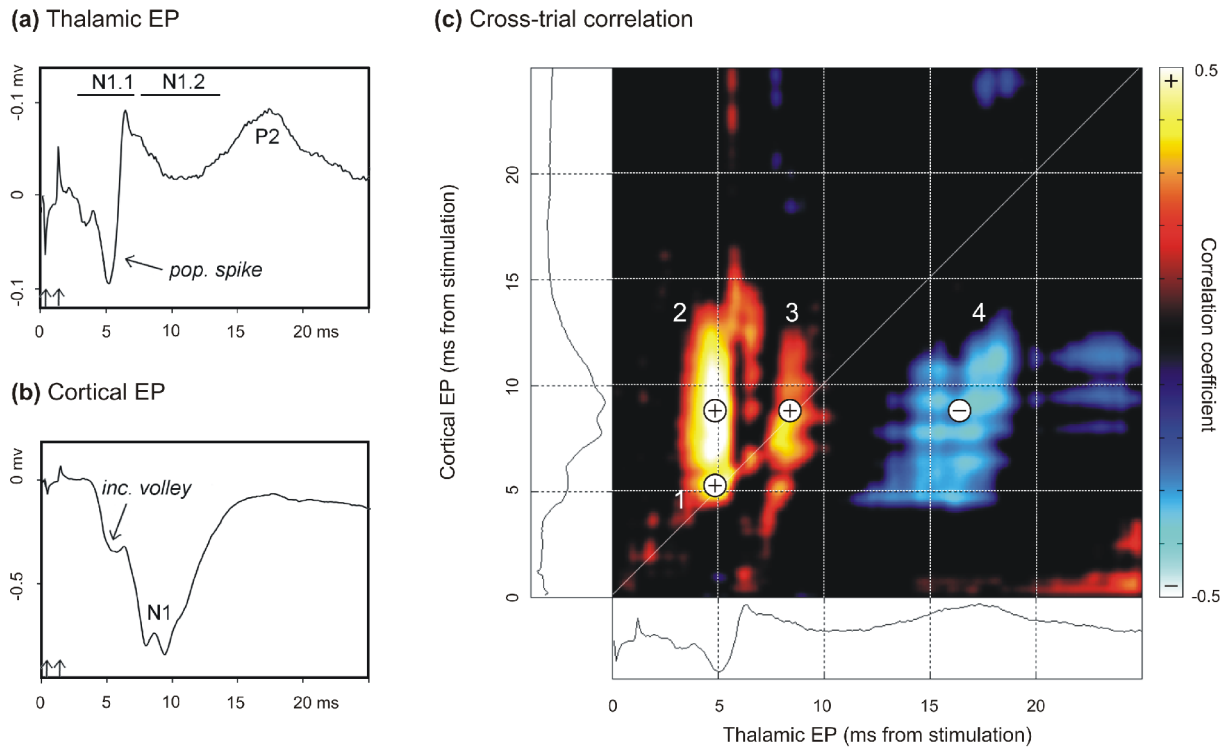

Fig. 2. Early waves of somatosensory evoked potentials recorded in control conditions from a pair of electrodes in one of the rats (panels (a) and (b)) and their cross-trial correlation matrix in (c). Small vertical arrows on the abscissa at (a) and (b) mark the on- and off-set of the 1 ms whisker stimulus. Corresponding recording artifacts can be seen in the traces. Panel (a) shows the average thalamic EP (n=100) from one of the electrodes located in VPM; N1.1 and N1.2 - components of early thalamic activation as identified in Kublik et al. 2003, pop.spike – population spike; P2 - thalamic inhibition (see text). Panel (b) shows the average cortical EP (n=100) from infragranular electrode; inc. volley. - incoming volley of thalamo-cortical fibers; N1 – activation of pyramidal cells. Panel (c) shows cross-trial correlation matrix calculated from 100 thalamic and 100 cortical EPs. Each pixel shows the significant coefficient value (non-significant coefficients were substituted with zeros) in color scale (colorbar on the right) for the latency combination that can be read from the time axes below and to the left of the matrix. A mean thalamic and cortical EP trace is also added below and on the left of the matrix, respectively, for orientation and viewing convenience, serving to easily relate correlation clusters to EP waves (latencies) which generate them. Four clusters of significant correlations are marked with numbers 1-4 (for details see text). The correlation matrix was smoothed with a 2-dimensional Gaussian kernel of 1 ms radius.

Results

Evoked potentials

A thalamic evoked potential in an anaesthetized rat starts around 3 ms with a negative wave deflection that consists of two subcomponents: N1.1 and N1.2 (Kublik et.al 2003). In an awake animal a multi-whisker stimulation evokes a sharp wave reaching its minimum at around 5 ms just after the first negativity of the N1.1 component (Fig. 2(a)). This wave is much weaker (if at all present) in anaesthetized rats (compare Fig. 3 in Kublik et al. 2003; and Fig. 3(a) in Łęski at al. 2009 - in this issue of JCN), as well as in non-anaesthetized rats after

single whisker stimulation (authors' unpublished observations). We believe that this prominent component recorded in awake rats reflects population spike activity evoked by strong, well synchronized multi-whisker input. Just after the population spike the second thalamic negative component N1.2 develops (at 7-14 ms), followed by a positive wave P2 with maximum at around 17 ms (Fig. 2(a)). As this wave is paralleled by a decrease in multi-unit activity (own unpublished observations) we believe that it reflects a diffuse inhibitory input to the thalamus.

Thalamic population spike (Fig 2 (a)) is further observed as an incoming volley at the earliest phase of cortical potential recorded by infragranular electrodes (Fig. 2 (b)), which directly proceeds the most prominent cortical response - a negative N1 wave. The N1 reaches its minimum at around 8-10 ms, lasts until ~15 ms and contains components reflecting, slightly shifted in time activations of two pyramidal cells populations from supra- and infragranular layers (Musiał et al., 1998; Kublik et al., 2001).

Thalamo-cortical cross-trial correlation for control conditions

The results of cross-trial correlation analysis for control conditions are presented in Fig 2(c) (example for single electrode pair in one of the rats) and Fig. 3(a) (group average, 7 electrode pairs). In these results, we identify 4 distinct clusters of significant correlation, labeled with numbers 1 through 4, according to chronology of the thalamic time scale. Since we selected the electrode pairs on a basis of highest correlation at the earliest thalamo-cortical connection, the respective cluster 2 was strongest, and present in each of individual correlation matrices. Subsequent clusters 3 and 4 were observed also in most (6 out of 7, and 5 out of 7, respectively) rats. Note that, as follows from the presentation of the cross-trial correlation method (see Methods), the clusters above the diagonal line correspond to thalamo-cortical influences and below the diagonal line - to cortico-thalamic influences. Simultaneous or near-simultaneous correlated events form clusters lying on the diagonal – like positive correlation cluster 1 at ~5 ms latencies in both thalamus and cortex (Fig. 2(c) and 3(a) and (b)).

This cluster 1 reflects the correlation of the thalamic population spike with the matching incoming volley recorded in the cortex. The highest mean correlation coefficients in this area reached 0.54±0.05 SEM. The following cluster 2 represents a positive correlation between the thalamic population spike and consecutive activation of pyramidal cells which generate the cortical N1 wave running from 6 to 13 ms. The highest mean correlation coefficients in this area reached 0.36±0.05 SEM. Cluster 3 encompasses positive correlations between subsequent early EP waves (at ~8 ms in the thalamus, 8-15 ms in the cortex). The highest mean correlation coefficients in this area reached 0.24±0.06 SEM. Cluster 4, on the cortico-thalamic side of the correlation matrix corresponds to the negative correlation of the main cortical activation (N1) and thalamic inhibition (P2) and most probably represents an indirect, cortical influence on the basolateral thalamus *via* inhibitory cells in the thalamic reticular nucleus (Landisman and Connors 2007). The lowest mean correlation coefficients in this cluster reached -0.27±0.07 SEM. To rule out any possibility that cluster 4 is an epiphenomenon caused by intrinsic thalamic EP dynamics we additionally calculated an auto-cross-trial correlation matrix for thalamic EPs (not shown) and found that there was no negative correlation between the population spike and P2 wave.

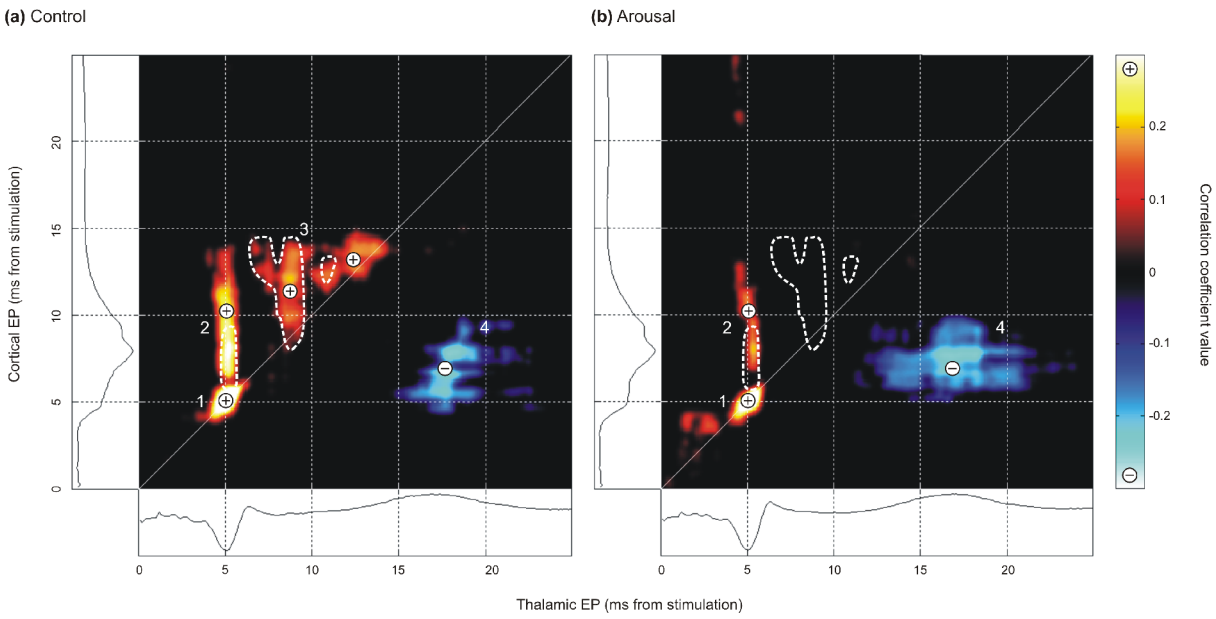

Fig. 3. Cross-trial correlation matrices averaged for a group of 7 rats (7 electrode pairs). Panel (a) shows results for control conditions; panel (b) - arousal conditions. Non-black pixels represent the coefficients that were significantly different from zero ($p<0.05$, t-test, $n=7$). White dashed white lines outline the regions of significant (paired t-test, $p<0.05$, $n=7$) differences between control and arousal data. The correlation matrix was smoothed with a 2-dimensional Gaussian kernel of 1 ms radius.

Effect of arousal

The cross-trial correlation analysis was repeated for the arousal conditions EPs recorded from the same seven electrode pairs. The group average result is presented in Fig. 3(b). The areas of significant differences ($p<0.05$, paired t-test, $n=7$) between both control and arousal conditions are outlined by dashed lines. It is clear from comparison of Figs. 3(a) and (b) that the positive correlation cluster 2 decreased with arousal. (The highest mean correlation coefficients in this cluster for arousal conditions reached 0.25±0.06 SEM, compared to 0.36±0.05 SEM for control conditions.) This posits the reduction of the thalamic impact on early cortical activation. Similarly, the positive correlation cluster 3 in Fig. 3(a) disappeared with increased level of arousal. We have not found a significant change of the negative correlation cluster 4, i.e. negative correlation between cortical activity (N1 wave) and thalamic positive (presumably inhibitory) P2 response. However, it is worth to note that at a more lenient significance threshold ($p<0.1$), this inhibitory correlation cluster has a significant tendency to shift to somewhat earlier latencies measured in the thalamus, suggesting that in an aroused state this circuit might operate in a more synchronized manner.

Note, that changes in cross-trial correlation (which is a normalized measure) between experimental situations are - in principle - independent of changes in average evoked potential amplitudes in those situations. In practice, in case of noisy recordings, smaller amplitude of EPs could possibly cause a noticeably greater proportion of signal variability being attributable to noise, whereupon EPs would be less outstanding in the noise, which in turn could lead to an artificially decreased correlation. Similar effect could be caused by smaller variability, and this should be kept in mind if using cross-trial correlation.

Therefore, we additionally verified whether the effect of the positive correlation cluster 2 decreasing with arousal could partially be such an epiphenomenon of the cross-trial

correlation. However, this was not the case. The mean cortical N1 amplitude did not change significantly between control and arousal conditions (paired t-test, $p>0.5$), while the amplitude of the thalamic population spike even increased (paired t-test, $p=0.001$) across all rats. (This latter effect mentioned here will be a subject of another paper.) There was no significant change either in the variability of the amplitude of the thalamic population spike or the cortical N1 wave (paired t-test, $p>0.5$).

Discussion

In this paper we describe a correlation method of inferring functional relations between interconnected brain structures from evoked local field potentials. The method measures how postsynaptic potentials (embedded in LFP) in one structure are shaped by activity in connected location(s). The method proved to be especially useful in investigating the relative changes of connections in a thalamo-cortical system at different functional states. With the results presented here we were able to show that relative thalamic impact onto cortical activity is decreased during contextual arousal (induced by aversive stimuli), compared to the awake, but habituated, quiescent state.

From the mathematical point of view the proposed cross-trial correlation method has the same limitations as the standard correlation between time-courses of two LFP signals. For instance, it would be difficult to distinguish between correlations produced by direct influence, common input or recurrent connections. In simple cases, like in our experimental example, this particular limitation is not relevant, because the wiring of the system we study is reasonably well known (Waite 2004, Kublik 2003). Our main goal, however, contrary to other connectivity studies (e.g. Korzeniewska et al. 2008), was not to discover which of the many recorded structures possibly influence other areas, but to quantify, in different contextual situations, the dynamics of activity flow between two structures comprising one sensory tract.

In more complex cases a way to differentiate between feedback and recurrent connections could be through analysis of the cross-trial autocorrelation, in addition to the correlation between the structures (see Fig. 1(d)). If we assume that noise present at every synaptic connection introduces distortions in the signal then we expect (in the feedback situation) higher values of cross-trial correlation between the structures than the cross-trial autocorrelation in the first stimulated structure. We can see this effect in the artificial data presented in Fig. 1(d), as well as in real data for the cortico-thalamic inhibitory correlation cluster 4: i.e. if we calculate an auto- cross-trial correlation matrix for thalamic EPs (not shown) there is no negative correlation between the population spike and P2 wave.

The cross-trial correlation method we propose can be obviously applied not only to the raw LFP signals. We expect that the method can be combined with other approaches. For instance in cases when one can extract signals describing activity of specific populations of cells (*via* e.g. factor analysis, Independent Component Analysis or other methods), the cross-trial correlations would be even easier to interpret as a direct measure of the strength of the connection between populations of cells.

We applied the newly developed method to rat somatosensory potentials evoked in the ventrobasal thalamus and in the infragranular barrel cortex, structures reciprocally connected by thalamo-cortical and cortico-thalamic fibers (for a review see Castro-Alamancos 2004). We wanted to observe the dynamics of neuronal connections between the two structures in awake rats at two behaviorally different states: during awake, but quiescent, well habituated

conditions (control) and during arousal induced by additional aversive stimulation. Our main observation was arousal-related attenuation of the correlation strength (coupling strength) of thalamo-cortical input (see cluster 2 in Fig. 3(a) and (b)), indicating that in awake animals an increase in arousal level is accompanied by a decrease of the participation of the thalamo-cortical input in shaping cortical activity. This contradicts the view according to which increased arousal intensifies thalamic influence on cortical activity (for reviews see Hasselmo 1995, Kimura 2000). The latter hypothesis is based mostly on experiments regarding action of certain arousal-controlling neuromodulators, mostly acetylcholine and noradrenaline, on synaptic and cellular properties of the cerebral cortex (Sillito and Kemp 1983, Sato et al. 1987, Gil et al. 1997). Increased release of these neuromodulators at certain cerebral locations, including the thalamus and cortex, leads to behavioral and electrophysiological signs of increased arousal (for reviews see McCormick 1993, Berridge and Waterhouse 2003). On the other hand, acetylcholine and noradrenaline were found to influence transmission at thalamo-cortical and cortico-cortical synapses, as well as excitability of cortical neurons, in such a way, that in the net cortical activity during arousal should strongly depend on external, in our case thalamic, input. It must be noted, however, that experiments supporting this view were performed mostly using *in vitro* techniques or iontophoretic application of pharmaceutical agents directly to the cortex.

The opposite effect was shown in behaving rabbits by Stoelzel et al. (2009). These researches found no state-related changes in the amplitude of average cortical field responses when thalamic spikes, with similar preceding interspike interval, were used as triggers for the spike-triggered averaging. This is in line with the results of Castro-Alamancos and Oldford (2002), who recorded evoked activity of the thalamus and cerebral cortex in anaesthetized rats, with and without electrical stimulation of the neuromodulatory centers of the brain stem reticular formation. In this model of arousal, thalamo-cortical flow of sensory information was attenuated by short term depression accompanying increased tonic activity of thalamic neurons. Similar conclusions were postulated by Castro-Alamancos (2004a) in a study on behaving rats, where the author compared rapid sensory adaptation in the barrel cortex during quiescent and task-oriented states.

Our data from behaving animals corroborate that the short term depression dominates over the neuromodulatory influences, which results in arousal-related attenuation of thalamic afferent impact onto activity of the primary somatosensory cortex. However it should be stressed, that the behavioral remodeling of thalamo-cortical activity reported here can differ among different cortical sensory-reactive areas (authors' unpublished observations), cortical layers (Wróbel et al. 1998, Kublik 2004) and stimulation pattern. Particularly, since there is no cross-whisker adaptation (Katz et al. 2006), the effects could be different if a single whisker stimulation was applied.

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