AMPLITUDE AND PHASE SYNCHRONIZATION IN A MODEL OF TEMPORAL LOBE EPILEPSY

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Abstract: In this study we investigated the reported phenomenon of a decrease in correlation between multisite electrophysiological recordings of human hippocampus during periods preceding ictal onsets in a model of temporal lobe epilepsy (TLE). We also examine a possible causal connection between the amplitude of neuronal oscillations and the level of phase coupling between spatially separated neuronal oscillators. Such connection can be relevant to distinguish between different seizure-generation mechanisms. One possible seizure onset scenario may be the formation of a compound, multi-site oscillator; an alternative scenario is related to dynamic synchronization between local oscillatory systems. We found that Gabor magnitudes in the high beta and gamma frequency bands became uncorrelated shortly before the seizure spreading process. However, local phase synchrony in broad bands monotonically increased shortly before and along with the seizure development. The epileptic discharges propagated bi-directionaly and with frequency-dependent phase delays between the septal and the temporal sites. Phase synchrony is generally related to the wavelet magnitude for frequencies in the gamma range in a unidirectional manner; increased magnitude leads to phase synchrony while the reverse association is statistically less pronounced.

Introduction

One distinctive feature of epilepsy is that it is a dynamic neurological condition [1,2]. Patients with epilepsy suffer from seizures that can appear at apparently irregular intervals, sometimes precipitated by external factors such as flickering lights, other times without any identifiable or reproducible cause. The exact chain of neuronal events leading to seizure (the ictal cascade) is in general unknown, although numerous animal and computer models have been proposed to explain the phenomena. From a practical point of view understanding the mechanisms leading to epileptic discharge can help in developing a methodology aimed at anticipation of seizures (for an extended methodological review see [3]) and possibly in designing strategies to prevent or abort epileptic seizures.

An electrographically feature related to epilepsy is the increased synchrony of electric activities measured during generalized seizures from different sites in the brain. Accordingly, our starting hypothesis in this study concerned the autonomous mechanisms of how such an extended oscillatory behaviour can originate. We can distinguish two basic scenarios. The first scenario (1) involves networks of bistable sub-systems each capable of oscillatory, limit cycle type of activity when activated. Such model networks have been built to describe cortico-thalamic circuits [4]. If by some noisy fluctuation or alternatively by an external perturbation one of those local networks gets excited and jumps into a limit-cycle type of dynamic mode, then it may ignite other bistable networks. The various oscillatory activities then can synchronize and spread even further. We consider this scenario as a generic model of secondary generalized seizures. In our second scenario (2) , no oscillatory units are present in the system. Instead, bursting units (integrate and fire), are connected in a dense network (every unit to every unit for example) and can only fire single shots with some refractory silent period after a discharge. If at a certain moment several units fire simultaneously due to noisy input, the whole system can start bursting continuously. Such seizure generation scenario is associated with the creation of a collective oscillator and we believe it can be a generic description of primarily generalized seizures.

To understand which type of scenario fits better one or another model of epilepsy, an analysis of EEG features characterising the amount of synchronization of the various rhythms can give important information. A temporary decrease of synchrony has been reported [5,6] in the pre-ictal phase of mesial TLE cases. Such a de-synchronization may favour the scenario of coupled non-linear oscillators rather than the collective oscillator model. In this study we analyse the evolution of synchrony of the neuronal oscillations as a system undergoes ictal transition. We consider here a broad range of measures of synchrony, some of which

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quantify the correlations between the magnitudes of the various oscillations, while others quantify the phase synchrony between the oscillatory sources. We also correlate the different measures of synchrony with the magnitudes of the oscillatory rhythms using non-stationary time-frequency techniques. This analysis is used to reveal the dynamic footprint of the epileptogenic process.

Materials and Methods

Dissection: For a full description, see [7]. Briefly, male C57/BL mice were anaesthetized with halothane and decapitated in accordance with the Canadian Animal Care Guidelines. The brain was extracted and placed in ice-cold, oxygenated standard ACSF. The hippocampus and septum were dissected out. Hippocampi were transferred to oxygenated room-temperature ACSF for a minimum of 1.5 hours before being placed in a dual perfusion input recording chamber (modified from [8]).

Experimental environment: Epileptiform activity was obtained by perfusing the tissue with low-Mg^{2+} ACSF (containing in mM: 123 NaCl, 5 KCl, 1.5 $CaCl_2$, 0.25 $MgSO_4$, 25 $NaHCO_3$, 1.2 NaH_2PO_4 and 15 glucose). Humidified warmed oxygen flowed over the solution in the chamber, maintained at 32 °C. ACSF entered the dual-input chamber both from the top and bottom of the tissue, fully surrounding the tissue with oxygenated solution.

Multisite electrophysiological recordings: Recordings were obtained from the CA1 cell layer of the hippocampus at four sites along the septotemporal hippocampal axis. We used a four-channel multiple electrode array, with an inter-electrode distance of 300 µm and an inter-pair distance of 1500 µm. Data were acquired using a custom-made four channel DC differential amplifier with an 8-pole Bessel lowpass filter (700 Hz), digitized at 2 kHz by a Digidata 1322 (Axon Instruments, Union City, California).

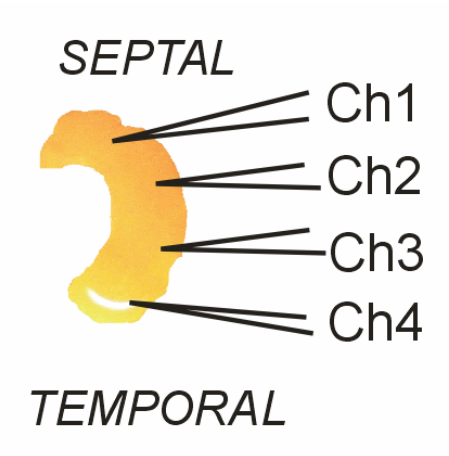

Figure 1: Schematic view of the electrodes positions and the associated data channels.

Gabor time-frequency representation. For each electrophysiological trace F(t) we define its Gabor time-frequency representation as

$$G(t,\nu) \equiv \int_{t'} dt'\, g(t-t',\nu) F(t') \qquad (1)$$

The Gabor aperture functions are given as

$$g(t-t',\nu) = \frac{1}{N_\nu} e^{-\nu^2 (t-t')^2 - i\alpha 2\pi\nu(t-t')} - O_\nu \qquad (2)$$

where the normalization and the offset factors N and O are chosen so that the functions have zero mean and unit quadratic norm. To quantify the correlations between two traces $F_1(t)$ and $F_2(t)$ we apply one of the following measures to the corresponding complex Gabor amplitudes $G_1(t,\nu)$ and $G_2(t,\nu)$.

Non-linear association index. A measure of monotonic functional dependence between two time series is given by the non-linear association index [9]

$$h^2(X\,|\,Y) = 1 - \sum_g \mathrm{var}_t(X(t)\,|\,Y(t)=g) \Big/ \mathrm{var}_t(X(t)) \qquad (3)$$

that compares the total variation of the signal X to the conditional variation of the signal X given Y. Note that this quantity is asymmetric in its X,Y arguments. We apply the above definition for the magnitudes (absolute values) of the Gabor weights.

$$H_{12}(\nu) = h^2(|G_1(t,\nu)|, |G_2(t,\nu)|) \qquad (4)$$

Here the variations are computed from moving window segments with length scaled to the Gabor wavelength (16 wavelengths were chosen here) and with 50% overlap.

Cross-amplitude. To quantify the magnitude of two oscillators that are simultaneously active we use:

$$XA_{12}(\nu) = \sqrt{\left\langle |\, G_1(t,\nu) G_2(t,\nu)| \right\rangle_t} \qquad (5)$$

Here and below the average in time is performed over a moving window with length scaled to the Gabor wavelength (16 wavelength chosen for the results in this article) and with 50% overlap. The centre of the window is an additional time parameter omitted from the formula above. The cross-amplitude time-frequency spectrum is shown in the scale-space representation in figure 3.

Amplitude correlation function. The formula below generalizes the stationary time correlation function.

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$$R_{12}(\nu)=\frac{\left\langle\left|G_1(t,\nu)\overline{G_2(t,\nu)}\right|\right\rangle_t-\left\langle\left|G_1(t,\nu)\right|\right\rangle_t\left\langle\left|G_2(t,\nu)\right|\right\rangle_t}{std_t\left(\left|G_1(t,\nu)\right|\right)std_t\left(\left|G_2(t,\nu)\right|\right)} \quad (6)$$

Amplitude coherency function. Very similar to (5) but with a different normalization, this is the time-frequency generalization of the coherency function:

$$C_{12}(\nu)=\frac{\left\langle\left|G_1(t,\nu)\overline{G_2(t,\nu)}\right|\right\rangle_t-\left\langle\left|G_1(t,\nu)\right|\right\rangle_t\left\langle\left|G_2(t,\nu)\right|\right\rangle_t}{\left\langle\left|G_1(t,\nu)\right|\right\rangle_t\left\langle\left|G_2(t,\nu)\right|\right\rangle_t} \quad (7)$$

Phase clustering index (PCI). A measure of phase clustering between complex amplitudes has been introduced [10] and used for anticipation of epileptic discharges induced by intermittent light stimulation [11] and for determining the probability of epileptic seizures in cases of TLE [12]. Here we introduce the Gabor version of this measure:

$$PCI_{12}(\nu)=\frac{\left\langle G_1(t,\nu)\overline{G_2(t,\nu)}\right\rangle_t}{\left\langle\left|G_1(t,\nu)\overline{G_2(t,\nu)}\right|\right\rangle_t} \quad (8)$$

This index is a complex number with magnitude <=1. Values close to 1 indicate that both traces have a constant time phase difference for the given frequency. Low values of the |PCI| indicate non-correlated phases. Finally, the phase of the PCI gives the dominant relative phase between the two traces.

Results

Our first computation regards the pre-ictal signals. The question is whether the phenomenon of pre-ictal de-synchronization can be reproduced. In figure 2 we see that after initial coupling between the temporal and septal traces a gap in association is observed. Note the time delay between the temporal and the septal traces as the seizure progresses. Initially the temporal signal "drives" the septal (maximal h^2 values at positive time delays), later the two signals correlate maximally at zero delay.

To analyse the situation in more detail we applied the measures (4), (6), (7) and (8) to the same traces as in figure 2. Our set of Gabor functions was selected with logarithmically spaced frequencies from 4 to 200 Hz. The averaging window consisted of 16 wavelengths and the exponential factor α, representing the number of wavelengths within the filter's aperture according to (2), was chosen to be 1. The window overlap was always 50%. Figure 3 represents the evolution of the averaged septal-temporal cross-amplitude during the seizure. A non-stationary, distributed oscillatory activity with a major frequency component of around 20Hz appears at the beginning of the seizure and develops within 20sec. into oscillatory activity of lower dominant frequency (around 6Hz).

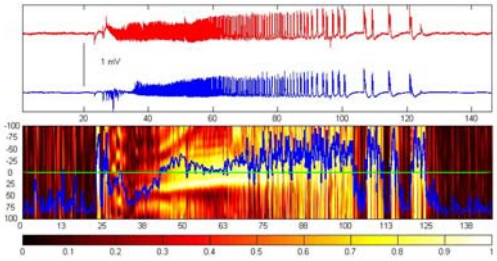

Figure 2: Non-linear association analysis between a septal and a temporal signal. Top plot shows the two signals. The time on the horizontal axis is in sec, the vertical axis represents the measured signal in mV. The bottom frame consists of a 2D plot showing the h^2 index between the two signals with a sliding window (centre indicated in sec. on the horizontal axis) and with mutual delay (in ms on the vertical axis, the horizontal green line marks the zero-delay border). For this analysis 1sec. of window length and 50% overlap was chosen. The blue trace indicates the maximum (over all the delays) of the h^2 index. Note the drop of the blue line at the beginning of the seizure indicates the association "gap" between the two oscillators.

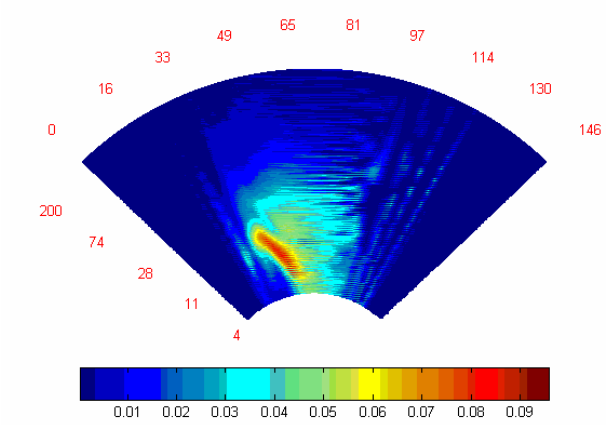

Figure 3: Scale-space representation of the averaged temporal-septal cross-amplitude (pseudo-color scale in mV). The radial axis represents frequencies in Hz and the azimuthally placed numbers are the centres of the scaled time windows in sec.

We see that in accordance with the overall h^2 analysis of figure 2 (the latter does not include frequency decomposition), the association "gap" is clearly visible on the top plot of figure 4. It occurs around the (electrographically detected) seizure onset (about 30 - 45 ms, see Fig 2) and in the gamma frequency band (20-70Hz). Further insight on this phenomenon can be obtained from the subsequent plots of figure 4. Both the amplitude correlation (R) and the amplitude coherence (C) functions show a change of

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sign within this time window indicating a switch from correlation to anti-correlation values. The bottom plot of figure 4 (PCI) shows, however, that the phase locking between the temporal and the septal signals is scarcely affected by the decrease of amplitude correlation. Quite the contrary, the PCI measure spreads across the frequency bands as the seizure evolves. Using the complex values of the PCI as given in eq. (8) we investigated also the relative phase between the temporal and the septal traces for the different frequency components as shown in Fig 5. From this figure it is clear that different rhythms show different phase order. For relatively low frequencies (up to 10Hz) the temporal signal shows an advancing phase while for the gamma band frequencies the phase sign is the reverse.

While figures 2-5 show the results of the analyses of one particular seizure (although very similar patterns were obtained for nearly all of the analysed 36 recordings) figure 6 displays an attempt to extract overall statistical information from all recorded seizures. We computed the association measure, again using again (3), between the different correlation quantities and the mean amplitude (A) and the cross-amplitude (XA) measures. The top plot of figure 6 presents the statistical result (mean and standard deviation) of $h^2(PCI, A)$, $h^2(A, PCI)$, $h^2(h^2, A)$ and $h^2(A, h^2)$ where A is the mean amplitude over all channels. The bottom plot presents the results from $h^2(PCI, XA)$, $h^2(XA, PCI)$, $h^2(h^2, XA)$ and $h^2(XA, h^2)$, where XA is the average cross-amplitude (eq. 4) for all temporal-septal pairs. We see clearly from figure 6, bottom plot that the cross amplitude XA explains the time variation of the PCI in the gamma band (approximately in the range 20-70Hz) in a unidirectional way. The same result, but less pronounced, can be observed for the associations with the averaged amplitude (top plot).

Discussion

The methodology used in this work is based on a non-stationary, Gabor wavelet type technique. Here we apply also a true scale-space type of window averaging technique where time windows are scaled with the wavelength of the Gabor filter members. We have chosen for window sizes equal to 16 wavelengths. Obviously, larger window sizes will produce better statistical confidence but at the same time it will challenge the assumptions of (quasi)stationary features of the signals within the chosen window. We analysed the results with different window sizes but the essential results were still valid.

The reason for using scaled sliding windows is to avoid statistical bias that might alter the results for the different Gabor wavelengths. Longer wavelengths will tend to correlate stronger than short wavelengths if taken within time windows with a fixed length.

We notice here again the non-symmetric nature of the association measure (3). In this article we exploit this property regarding the results presented in figure 6. We did not search for causality between two time series in the sense of temporal precedence but for relative determinism between couples of simultaneous measurements. If one signal can explain the variation of another signal but the second signal explains poorly the variation of the first one, we assume that there is a possible unidirectional connection. In other words, this situation reveals that the first signal can be sufficient but not a necessary factor for the values of second signal. The statistical results of figure 6 indicate that the dynamic scenario responsible for this *in vitro* model of TLE of seizures is most likely that of a coupled set of oscillators, each capable of generating ictal activity. When activated, these oscillators eventually synchronize their phases. The alternative, the collective oscillator scenario would imply high-amplitude oscillatory activities causally related to the phase synchrony. As the increase of phase synchrony in our case does not result directly in a change of oscillator's (cross) amplitude, but the change of the latter results in a change of the phase synchrony, we conclude that the "control parameter" in these cases is the amplitude rather than the synchronization between the oscillators. We note that this finding is in line with seizure prediction methodologies based on accumulated energy [13] which is another computational technique to study oscillatory amplitudes.

The speculated dynamic scenario also relates to our previous observations from MEG studies in cases of photosensitivity [11] and hippocampal recordings in patients with TLE [12] where the acute risk of seizure occurrence has been probed using external low-intensity electrical current stimulation paradigms. Periodic external stimulation is a source of induced oscillatory activity that can further reveal measurable features, such as the relative PCI, that are correlated to the probability of an instantaneous ictal transition.

Conclusions

To summarize, analysis of 36 seizures of low Mg model of temporal lobe epilepsy shows that:

- Wavelet techniques give an accurate picture of the ictal synchrony evolution.
- Non-linear association index as well as other amplitude based methods shows short-lasting pre-ictal de-correlation in specific frequency bands.
- Phase synchrony measured by PCI "bridges" the above pre-ictal gap.
- Phase relations between septal and temporal traces are frequency-dependent (independently found in Derchansky et al, submitted to *Brain*).
- Phase synchrony for frequencies in the gamma range is related to the wavelet magnitude in a unidirectional manner.
- Paradigms aimed at measuring or actively modulating the magnitude of neuronal oscillations by external

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stimulation might be advantageous when used for assessing the risk of ictal transitions.

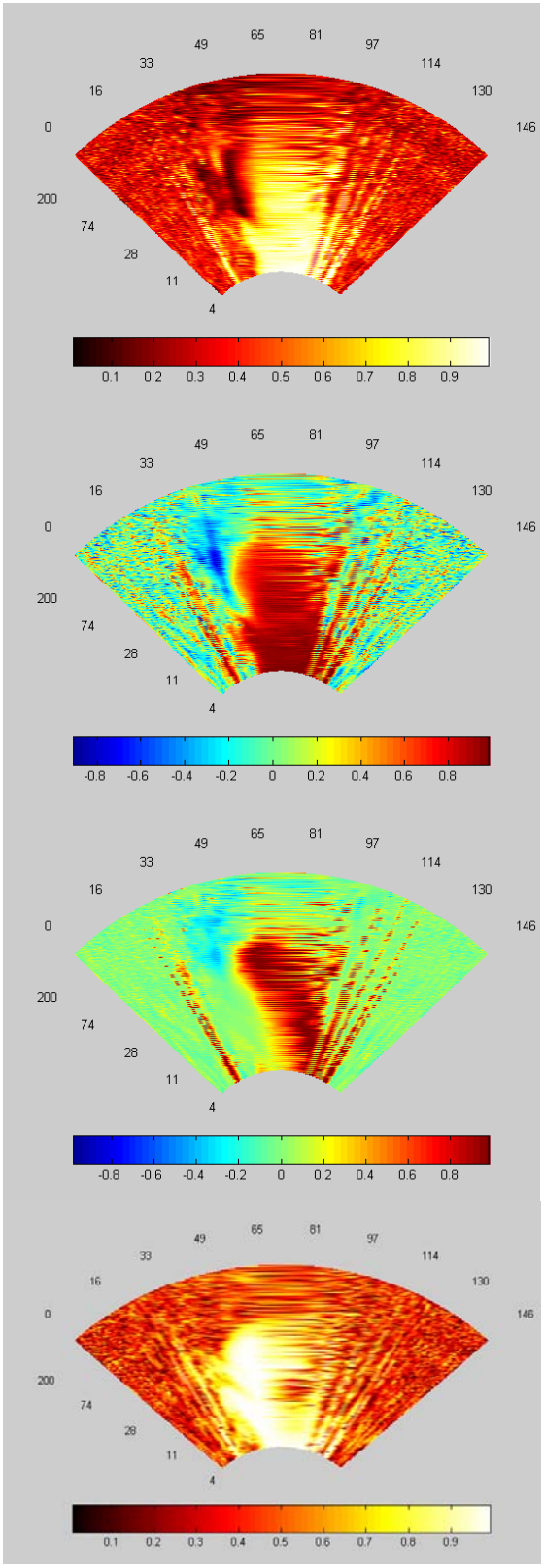

Figure 4: examples of the different signal features as described in **Materials and Methods**. From the op: the h^2 association computed between a septal and a temporal trace (channels 1 and 3 from figure 1); the R^2 amplitude correlation function; the C^2 amplitude coherency function and the PCI representing the phase clustering between the same traces. The notations of the scale-space plots are the same as in figure 3.

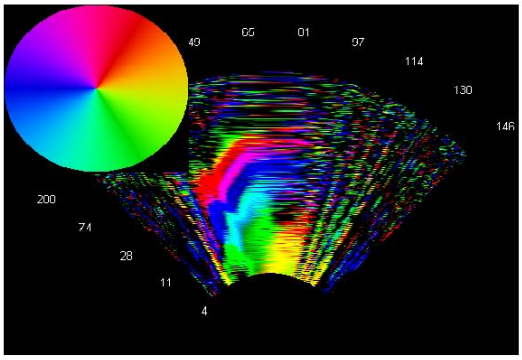

Figure 5: A scale-space plot of the septal-temporal relative phase evolution during a seizure. The pseudo-colour coding of the phases is given by the spectral circle in the top-left corner. The frequency and time notations are the same as in figures 3 and 4. We see a clear frequency dependence of the relative phase: while in lower frequency bands the green colour indicates temporal oscillations leading the septal oscillators (negative phase difference), in the gamma band the order is reversed.

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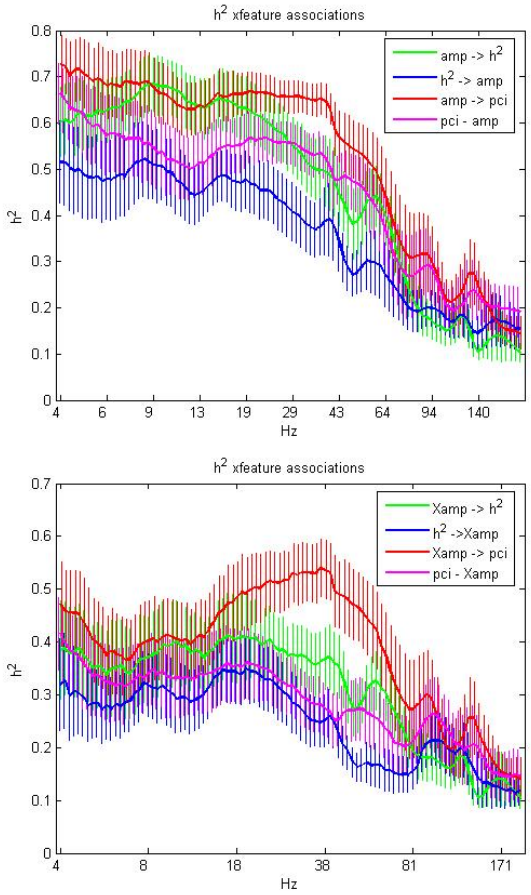

Figure 6: **S**tatistical analyses of the inter-feature associations. The lines represent the mean values of the non-linear associations between the different signal feature and the vertical bars represent the standard deviations. In total 36 seizure segments were analysed. In the top plot the averaged channel amplitudes were associated with the pair-averaged h^2 septal-temporal association measures (green and blue lines) and with the averaged PCI between all septal and temporal traces (red and magenta lines) .In the bottom plot the averaged cross-amplitude for all pairs of temporal-septal channels was used in place of the averaged amplitude. From the bottom plot we see that the cross-amplitude is unidirectionaly associated with the PCI (the red and magenta lines). Unidirectional association is also visible, but to a lesser degree, for the cross-amplitude and the h^2 septal-temporal association. The same conclusions are visible from the top plot where the averaged Gabor amplitude is unidirectionaly associated with the PCI.

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