Chapter 4

Fast network oscillations in vitro exhibit a slow decay of temporal auto-correlations

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Abstract

Ongoing neuronal oscillations in vivo exhibit non-random amplitude fluctuations as reflected in a slow decay of temporal auto-correlations that persist for tens of seconds. Interestingly, the decay of auto-correlations is altered in several brain-related disorders, including epilepsy, depression and Alzheimer’s disease, suggesting that the temporal structure of oscillations depends on intact neuronal networks in the brain. Whether structured amplitude modulation occurs only in the intact brain or whether isolated neuronal networks can also give rise to amplitude modulation with a slow decay is not known. Here, we examined the temporal structure of cholinergic fast network oscillations in acute hippocampal slices. For the first time, we show that a slow decay of temporal correlations can emerge from synchronized activity in isolated hippocampal networks from mice, and is maximal at intermediate concentrations of the cholinergic agonist carbachol. Using zolpidem, a positive allosteric modulator of GABA_A receptor function, we found that increased inhibition leads to longer oscillation bursts and more persistent temporal correlations. In addition, we asked if these findings were unique for mouse hippocampus, and we therefore analysed cholinergic fast network oscillations in rat prefrontal cortex slices. We observed significant temporal correlations, which were similar in strength to those found in mouse hippocampus and human cortex. Taken together, our data indicate that fast network oscillations with temporal correlations can be induced in isolated networks in vitro in different species and brain areas, and therefore may serve as model systems to investigate how altered temporal correlations in disease may be rescued with pharmacology.

Introduction

Neuronal oscillations are thought to play a critical role in the encoding and retention of information (Singer, 1999; Buzsáki, 2006; Michels et al., 2008; Palva et al., 2010). In humans, working memory studies have pointed to a functional connection between memory and the amplitude modulation of oscillations in different frequency bands (Jensen et al., 2002; Howard et al., 2003; Jokisch and Jensen, 2007; van Vugt et al., 2010). Notably, parietal oscillations exhibit systematically longer periods of elevated amplitude with longer encoding and retention intervals in a
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Sternberg task (Raghavachari et al., 2001). Thus, the temporal stability of oscillations may be important for mnemonic operations.

Preclinical studies have also pointed to a functional importance of the temporal structure of oscillations. For example, patients with early-stage Alzheimer's disease had less persistent temporal (auto-)correlations (1–25s) and less stable alpha oscillations on short-to-intermediate time scales (<1s) compared with healthy subjects (Montez et al., 2009). Altered temporal correlations without changes to time-averaged oscillation power have also been observed in other disorders and frequency bands (Linkenkaer-Hansen et al., 2005; Monto et al., 2007). Thus, identifying factors influencing amplitude fluctuations of oscillations could provide a better understanding of pathophysiological states and, possibly, yield new targets for treatment.

The hippocampus is a key neuronal structure for memory and cognition (Axmacher et al., 2006; Montgomery and Buzsáki, 2007), and it has been argued that gamma oscillations in the hippocampus play an important role in memory formation, because they serve the function of binding functional regions (Freund and Buzsáki, 1996; Fell et al., 2001; Lisman et al., 2005; Axmacher et al., 2006; Colgin et al., 2009).

Hippocampal network oscillations can be induced in vitro by muscarinic acetylcholine receptor activation (Fisahn et al., 1998). These oscillations fall in the beta-frequency range (15–30 Hz) when measured below 30 °C, but in the gamma range (30–100 Hz) if recorded at physiological temperatures (Dickinson et al., 2003). Here, we will refer to these oscillations as ‘fast network oscillations’ (Mann et al., 2005).

Fast network oscillations induced in vitro share many characteristics with gamma oscillations generated in CA3–CA1 in vivo (Csicsvari et al., 2003) and have been used as a model to identify cellular and synaptic mechanisms that shape the power, frequency or coherence of these oscillations (Bragin et al., 1995; Fisahn et al., 1998; Traub et al., 2000; Tiesinga et al., 2001; Csicsvari et al., 2003; Mann and Paulsen, 2005).

It is not known whether temporal (auto-)correlations are an exclusive feature of extended neuronal networks in vivo, or whether reduced neuronal networks in vitro may also exhibit these correlations, and what factors may shape their amplitude structure. To address these questions, we studied cholinergic fast network oscillations in vitro. We modulated cholinergic and GABAergic signalling to test how these neurotransmitter systems affect the amplitude structure of fast network oscillations. We found that temporal auto-correlations can emerge in acutely isolated hippocampal and prefrontal cortex brain slices. Interestingly, the temporal correlations were maximal at physiologically relevant levels of cholinergic drive, and could be enhanced by increasing inhibition.
Materials and methods

Slice preparation and local field potential recordings. Experiments were performed in accordance with the guidelines and with the approval of the Animal Welfare Committee of the VU University Amsterdam, which operates in accordance with Dutch and European law. Unanesthetized DBA/2J mice (19 slices, 10 mice, 5 males, Jackson Laboratories) and Wistar rats (n = 10, Harlan, The Netherlands) were decapitated at postnatal day 13–15 and postnatal day 14–28, respectively. Their brains were quickly removed and placed in ice-cold artificial cerebrospinal fluid (ACSF) containing (in mM): 125 NaCl, 25 NaHCO$_3$, 3 KCl, 1.2 NaH$_2$PO$_4$, 1 CaCl$_2$, 3 MgSO$_4$ and 10 D(+)-glucose (carbonated with 5% CO$_2$/95% O$_2$) (for rats: 1.25 NaH$_2$PO$_4$ and 26 NaHCO$_3$). For mice, horizontal slices (400 μm thick) from the ventral hippocampus were cut by a microtome (Microm, Waldorf, Germany). Slices were stored in an interface storage chamber at room temperature (22 °C) and placed in ACSF containing 2 mM CaCl$_2$ and 2 mM MgSO$_4$. After 1 h, slices were placed on 8 × 8 planar multi-electrode arrays (Titanium nitride electrode grids, electrode diameter 30 μm, contact impedance 30–50 kΩ, 200 μm distance, 60 recording electrodes; Multi Channel Systems GmbH, Reutlingen, Germany) (see Fig. 4.1A), with polyethylenimine coating (Sigma-Aldrich, St Louis, MO, USA), and left for 1 h in a chamber with humidified carbogen gas before their placement into the recording unit. The setup allowed for simultaneous measurements of four slices. The flow rate during recordings was 4–5 mL/min and the temperature was kept low (30 ± 0.3 °C) to preserve slice stability. The amplitude of oscillations at higher temperatures was also markedly lower than at 30°C, resulting in an unfavourable signal-to-noise ratio. Therefore, all experiments were performed at 30°C (Van Aerde et al., 2009). Zolpidem was purchased from Duchefa (Haarlem, The Netherlands) and carbachol from Sigma (St Louis, MO, USA). Local field potentials were recorded (Fig. 4.1E) at 1 kHz. The recordings were down-sampled off-line to 200 Hz and converted to MATLAB (The Mathworks, USA) files. Analysis was performed in MATLAB.
Figure 4.1 Carbachol (carbamylcholine chloride) (CCH) induces oscillations in hippocampal slices. (A) A multi-electrode array covering an entire hippocampal slice. Black dots are electrodes, which have a separation of 200 μm. This picture was made for every experiment, and used to classify the electrodes into the nine hippocampal sub-regions shown in B. (B) Schematic overview of the hippocampal slice divided into sub-regions of dentate gyrus (DG); stratum molecular, stratum granulosum and hilus region, CA1/CA3; stratum radiatum/lacunosum–moleculare, stratum pyramidale and stratum oriens. (C) Peak frequency fitting method. A 1/f spectrum (thin red line) is fitted to the power spectrum (Welch method) (thick blue line), and the confidence interval is determined (dashed green line). If the peak is above the confidence interval, the peak frequency, power and width are estimated by fitting a Gaussian to the power spectrum subtracted with the 1/f spectrum (see Materials and methods). (D) Power spectrum (Welch method). We studied oscillations in the dominant frequency range. (E) Visual inspection of local field potential (LFP) signals reveals that CCH induces oscillations. Examples of filtered LFP signals (10–40 Hz) at different concentrations from one channel in CA3 stratum radiatum/lacunosum–moleculare. (F) Grand-average multi-taper time-frequency plot shows the main frequency of the oscillations. Moving window size 1024 points (3.7–47.9 Hz). (Top) CCH experiment. Median across 14 DBA/2J mice in CA3 stratum radiatum/lacunosum–moleculare, one channel per slice. (Bottom) Zolpidem experiment. Median across 11 DBA/2J mice in CA3 stratum radiatum/lacunosum–moleculare, one channel per slice.
For rats, coronal sections (350–400 μm) of the prefrontal cortex were cut using a Leica VT1000S vibratome slicer. Slices were then transferred to holding chambers in which they were left to recover at room temperature for 1 h in ACSF containing (in mM): 1.25 NaH$_2$PO$_4$, 2 MgSO$_4$, 2 CaCl$_2$ and 26 NaHCO$_3$ (see (Van Aerde et al., 2009) for more details). After recovery, slices were mounted on 8 × 8 arrays of planar Indium tin oxid microelectrodes (electrode size 50 × 50 μm, electrode impedance < 22 kΩ, inter-polar distance, 150 μm; Panasonic MED-P5155, Tensor Biosciences, Irvine, CA, USA). Multi-electrode probes were coated with 0.1% polyethylenimine (Sigma-Aldrich) in 10 mM borate buffer (pH 8.4) for at least 6 h before use. The multi-electrode probe was then placed in a chamber saturated with humidified carbogen gas for at least 1 h. For recordings, slices were maintained in submerged conditions at 25 °C and superfused with ACSF, bubbled with carbogen, at 4–5 mL/min. Spontaneous field potentials from all 64 recording electrodes were acquired simultaneously at 20 kHz, using the Panasonic MED64 system (Tensor Biosciences), and down-sampled off-line to 200 Hz. Slices were recorded for a minimum of 10 min with 25 μM carbachol.

**Hippocampal concentration curves.** During each recording session, four slices were recorded simultaneously. After placing the slices in the recording units with ACSF, spontaneous activity was recorded for 15–20 min. Subsequently, two different experimental protocols were followed. For the first protocol, the following concentrations of carbachol were washed onto the slice: 1, 1, 5, 10, 15, 20 and 25 μM. For each concentration, the activity was recorded for 20 min. For the second protocol, to measure the effect of GABAergic modulation, we used the following concentrations of carbachol and zolpidem, and the activity was recorded for the specified duration: 5 μM carbachol, 0 μM zolpidem, 45 min; 5 μM carbachol, 0 μM zolpidem, 25 min; 5 μM carbachol, 100 nM zolpidem, 15 min; and 5 μM carbachol, 1 μM zolpidem, 20 min. The timelines of these experiments are shown in Fig. 4.1F.

**Hippocampal slice selection and preanalysis.** For each experiment, a photograph was taken of the slice in the recording unit in order to identify electrode locations (Fig. 4.1A). The hippocampus consists of three main regions: CA1, CA3 and the dentate gyrus. We divided CA3 and CA1 into the sub-regions stratum oriens (basal dendrites), stratum pyramidale (cell bodies) and stratum radiatum/lacunosum–moleculare (apical dendrites), and the dentate gyrus into stratum molecuare (basal dendrites), stratum granulosum (cell bodies) and hilus (apical dendrites)
(Fig. 4.1B). We developed an interactive MATLAB procedure to classify each electrode in one of these nine hippocampal sub-regions, based on the picture of the electrode grid on the hippocampus. Using Fourier analysis (see below), we determined for each channel whether oscillatory activity was present. Channels with no clearly detectable peak and, thus, a very low signal-to-noise ratio were excluded from further analysis (<5% of the data).

**Peak frequency fitting and preprocessing of data.** We developed an algorithm to objectively determine the peak frequency of the dominant oscillation (Fig. 4.1C). First, we computed the power spectrum using the Welch method (Fig. 4.1C, *thick blue line*), and determined the approximate frequency interval at which the peak occurred by visual inspection in the 10–25 Hz range, e.g. from 14 to 18 Hz in Fig. 4.1C. The median selected frequency range was 15–23 Hz. If no clear peak was seen, a standard interval of 10–25 Hz was chosen. A $1/f$ line was fitted to the power spectrum in the interval from 2 to 43 Hz, excluding the peak interval (Fig. 4.1C, *thin red line*). The $1/f$ power spectrum was subtracted from the original spectrum, and a Gaussian was fitted in the interval defined by the visual inspection to find the peak frequency and peak power. We calculated the power as the median of the peak power minus the $1/f$-fitted baseline power (Fig. 4.1C), across channels in each sub-region. Peaks were excluded from peak frequency/power analysis if: (i) the peak power was below the 95th confidence interval (Fig. 4.1C, *dashed green line*) of the $1/f$ fit, (ii) the peak was outside the 10–25 Hz interval, or (iii) the peak width was < 0.5 Hz. This method is similar to the one used in (Jansen et al., 2009). Note that in vitro experiments performed below physiological temperatures lead to prolonged GABAergic inhibitory postsynaptic currents and we therefore observed peak frequencies at lower frequencies than have typically been reported at higher temperatures (see Supporting Information Fig. 4.51) (Bragin et al., 1995; Dickinson et al., 2003). Importantly, the frequencies found in our study are similar to those reported in similar studies (Fisahn et al., 2009; Jansen et al., 2009; Van Aerde et al., 2009).

As reported previously (Mann and Paulsen, 2005; Jansen et al., 2009), the dominant frequency component of carbachol-induced oscillations is in the interval 10–25 Hz (Fig. 4.1D). Here, we only analysed the dominant frequency component, because higher frequencies were either harmonics of this dominant frequency or of very low signal-to-noise-ratio, which does not allow for the detection of temporal correlations (Linkenkaer-Hansen et al., 2007).
Amplitude envelope. The amplitude envelope of the oscillations was extracted using band-pass filters (finite impulse response filters with a Hamming window) and the Hilbert transform (Fig. 4.2B). The band-pass was centred at the peak frequency and had a width of \(0.5\sqrt{2}\) SD [filter order 60, SD is the width of the Gaussian fit to the peak (Fig. 4.1C); a 10–25 Hz band-pass was used if no peak frequency was found]. The median frequency range was 10–25 Hz. For the rat data only a 10–25 Hz band-pass was used, because analyses were also performed on channels not having a clear frequency peak.

Rejection of high-amplitude artefacts. We performed a rejection of high-amplitude artefacts in the amplitude envelope before oscillation burst analysis and detrended fluctuation analysis (DFA) (see below). This is recommended, because high-amplitude artefacts can corrupt the temporal structure and estimates of temporal correlations, whereas the removal of small segments of the time series has a negligible influence on the DFA exponent of a time series (Chen et al., 2002). Our artefact-rejection algorithm examined the amplitude envelope twice. In the first run, a window of 2 s was removed around amplitude values larger than 6 standard deviations from the median of the entire amplitude envelope and replaced with a zero. In the second run, the algorithm removed a window of 1 s around large-amplitude artefacts that were larger than 6 standard deviations.

Oscillation burst structure analysis. The life-times of oscillation bursts were based on the amplitude envelope. We defined an oscillation burst to begin when the amplitude envelope passed above a predefined threshold (determined in time windows of 1 min, to compensate for any slow transients in the level of activity), and to end when the amplitude envelope passed below this threshold (Fig. 4.2B) (Poil et al., 2008). The life-time measure for a signal was then determined as the 95th percentile in the cumulative probability distribution of the life-times (Fig. 4.2C). We systematically mapped the influence of different thresholds (Fig. 4.2D). The results were largely independent of threshold and therefore we only report statistics for the 0.5*median threshold.
Figure 4.2 Temporal correlation and oscillation burst life-time analyses characterize the non-random temporal structure of amplitude fluctuations in oscillations. 

(A) The study of spatial and temporal dimensions of neuronal processing requires different correlation analyses. Coordination of anatomically distributed activity (parallel processing) may be studied by computing correlations between neuronal signals from different hippocampal regions (Cross-correlations). In contrast, coordination of brain activity over time (serial processing) may be studied by computing temporal auto-correlations in neuronal signals within a single hippocampal region (Auto-correlations). Serial processing requires a sequence of causally related neuronal activities, which is likely to give rise to correlations over time (temporal correlations), e.g. persistent oscillatory activity as reflected in a slow amplitude modulation as studied here. Thus, by studying auto-correlation properties we may learn about novel mechanisms of attention and memory. Figure from (Montez et al., 2009) ©2009 National Academy of Sciences, USA. 

(B) The raw signal was band-pass filtered around its peak frequency (thick blue line), and the amplitude envelope (thin red line) was extracted using the Hilbert transform. To quantify differences in oscillation burst dynamics on short to intermediate time scales (< 1 s), we introduced a threshold at multiples of the median amplitude envelope (exemplified by the median threshold, top horizontal dashed line, black areas) and 0.5*median amplitude (lower horizontal dashed line, black and blue areas), and defined the start and end of an oscillation burst as the time points of crossing this threshold. The oscillation burst structure is largely independent of which threshold is used. 

(C) Cumulative probability distribution plot of life-times calculated using 0.5*median as the threshold at different concentrations. 

(D) Three-dimensional plot showing the 95th percentile life-time vs. carbachol (carbamylcholine chloride) (CCH) concentration, normalized with the life-time at the first period with 1 μM CCH, for different thresholds. We tested thresholds in the range of 0.1–1.1 times the median amplitude envelope. We normalized the life-time to avoid displaying the trivial effects of increased life-time with lower threshold. We observed that the life-time is largely independent of which threshold is used. 

(E) Amplitude envelopes of a signal with a low DFA exponent (0.52; 1 μM CCH, blue trace), a high DFA exponent (0.65; 10 μM CCH, red trace), and an
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intermediate exponent (0.55; 20 μM CCH, *green trace*). Visual inspection of the amplitude modulation at different CCH concentrations reveals a more stable modulation (high DFA exponent, long life-time) at physiologically relevant concentrations (15 μM) compared with higher CCH concentrations. Example segments of the amplitude envelope from the same slice at different CCH concentration. (F) Visualization of DFA for a signal with a low DFA exponent (0.52; *blue circles*), an intermediate exponent (0.55; *green squares*) and a high DFA exponent (0.63; *red plus*es). When the signal with high DFA is shuffled in oscillation cycle wide windows, the correlations disappear (0.50, *black dots*). The DFA exponents are the slopes of the lines obtained with linear regression in log–log coordinates. The DFA plots correspond to the amplitude envelopes partially shown in E. (G) Auto-correlations of the amplitude envelopes in E [1 μM CCH (*blue circles*), 20 μM CCH (*green squares*) and 10 μM CCH (*red plus*es)]. When the amplitude envelope of the 10 μM CCH is shuffled in oscillation cycle wide windows, the correlations disappear (*black dots*, Supporting Information Fig. 4.S5).

**Detrended fluctuation analysis of temporal auto-correlation decay.** The DFA is a method used to quantify the decay of temporal (auto-)correlations in time series with less strict assumptions about the stationarity of the signal than the classical auto-correlation function or power spectral density (Peng et al., 1994). An additional advantage of DFA is the greater accuracy in estimating the decay of auto-correlations from a limited amount of data (Gao et al., 2006; Linkenkaer-Hansen et al., 2007). Here, we used the DFA to quantify the complex temporal structure of amplitude fluctuations in ongoing oscillations in the time range of 3–20 s. We note that previous studies have applied DFA on similar time scales and used the DFA exponents to show that the temporal structure of ongoing oscillations differs between males and females (Nikulin and Brismar, 2005), isheritable (Linkenkaer-Hansen et al., 2007) and is sensitive to disease (Montez et al., 2009). Together, these findings in humans underscore the relevance of quantifying auto-correlations in ongoing oscillations on time scales up to a few tens of seconds using DFA.

The main steps from the broadband signal to the quantification of temporal correlations using DFA have been explained in detail elsewhere (Linkenkaer-Hansen et al., 2001). In brief, the DFA provides a measure of how the root-mean-square fluctuation of the integrated and linearly detrended signals, \( F(t) \), scales as a function of time window size, \( t \) (Fig. 4.2F). We computed the fluctuation function, \( F(t) \), with an overlap of 50% between windows. The DFA exponent is the slope of the fluctuation function. A DFA exponent in the interval of 0.5–1.0 indicates the presence of temporal (auto-)correlations, whereas an uncorrelated signal is characterized by an exponent of 0.5. The temporal correlations are not computed between areas, but within the signal (auto-correlations) (Fig. 4.2A). In Fig. 4.2E and F it is shown that the high DFA exponent at 15 μM in Fig. 4.2F is qualitatively reflected in amplitude modulation on long
time scales compared with lower or higher concentrations of carbachol (Fig. 4.2E). The correspondence between the DFA and the classical auto-correlation function is shown in Fig. 4.2F and G using the representative signals from Fig. 4.2E. As reported earlier, however, we found that the auto-correlation function was too noisy for estimating the decay of correlations (Gao et al., 2006; Linkenkaer-Hansen et al., 2007).

**Statistical analysis.** Biomarkers obtained from the analysis of the oscillations were tested for normality using Lilliefors’ composite goodness-of-fit test and were often observed to not follow a Gaussian distribution. As an example of this we show distributions in Supporting Information Fig. 4.S4. Therefore, we used non-parametric tests. To test for the effect of the carbachol or zolpidem concentration on frequency, power, life-time or DFA exponents, we used the non-parametric Friedman test (with F statistics) (Friedman, 1937; Conover and Iman, 1981). In our case, the Friedman test tests for paired differences in the median between observations at different concentrations of carbachol or zolpidem. For example, in an interval from 10 to 25 μM, it would test for paired differences in the median between the observations at 10, 15, 20 and 25 μM. For the Friedman test, a Greenhouse–Geisser correction was used to correct for sphericity when the Greenhouse–Geisser epsilon was below 0.75, and a Huynh–Feldt correction was used otherwise (Greenhouse and Geisser, 1959; Huynh and Feldt, 1976). When comparing the medians of two groups, such as two concentrations, we used permutation tests on the median, with more than 10.000 permutations (Box and Andersen, 1955; Ernst, 2004). Figures 3A, 4B, and 5A show life-times normalized with the life-times from the first 1 μM carbachol measurement; nevertheless, all tests were performed on non-normalized data. Box plots were used to display the distribution of data; the boxes have lines at the lower quartile, median (red line) and upper quartile values. The whiskers are lines extending from each end of the boxes to show the extent of the rest of the data (up to 1.5 × interquartile range of the sample) (McGill et al., 1978). Confidence intervals were found using non-parametric bias corrected and accelerated bootstrap (BCa) (DiCiccio and Efron, 1996). Note that we used paired data for the statistical tests on the effect of concentrations, and therefore the confidence intervals cannot be used to infer significance. We used Holm’s step-wise Bonferroni correction for multiple comparisons within single measures when appropriate (Holm, 1979), and binomial correction when the power of Holm correction was too low (defined as when more than one sub-region had P-values below 0.05, but all sub-regions were rejected by Holm correction). Binomial correction only controls the Type 1 error
at the number of significant sub-regions. Significance was defined as $P < 0.05$ for all tests.

**Results**

The frequency and power of cholinergically-induced fast network oscillations in hippocampal slices *in vitro* strongly depend on the activity of pyramidal cells and interneurons in the CA3 area (Traub et al., 2000; Mann and Paulsen, 2005; Heistek et al., 2010). Typically, cholinergic receptor activation induces oscillations in the CA3 and CA1 area with the strongest power in CA3 but with similar frequency. It is not known whether amplitude fluctuations of these oscillations have a non-random temporal structure. Therefore, we recorded local field potentials from hippocampal slices using 8×8 multi-electrode grids with 200 μm spacing that covered the transverse section (Fig. 4.1A and B). Fast network oscillations were induced by application of the muscarinic acetylcholine receptor agonist carbachol, and field potential oscillations in the different hippocampal areas were analysed for peak frequency (Fig. 4.1C), power, burst duration and temporal correlations using DFA. We quantified the duration or ‘life-time’ of oscillation bursts by the heavy tail of their probability distribution using the 95th percentile (Fig. 4.2B–D). In Figs. 4.3 and 4.5, all sub-regions in which significant effects were observed are marked with black dots. In the following we will for simplicity only comment on observations from sub-regions in CA3.

**Oscillation burst life-time has a bell-shaped dependence on carbachol concentration.** To test whether the temporal structure of oscillations is dependent on the level of cholinergic activation, we applied increasing concentrations of carbachol (Fig. 4.1F). First, spontaneous activity was recorded for 15–20 min in the absence of carbachol. Subsequently, carbachol was bath applied in concentrations of 1, 5, 10, 15, 20 and 25 μM. For each concentration, the activity was recorded for 20 min. The life-time of oscillation bursts increased with increasing carbachol concentration (1–10 μM) and, interestingly, decreased when the carbachol concentration was further increased (10–25 μM) in all sub-regions of CA3 (Fig. 4.3A), e.g. from 1600 ± 680 ms (median ± 95% BCa confidence interval halfwidth) to 1220 ± 340 ms in stratum radiatum/lacunosum–moleculare of CA3 (Fig. 4.3A). To test the significance of the apparent bell-shaped dependence of life-time on carbachol concentration, we tested for a difference in the median with one-tailed paired permutation tests between 5 and 15 μM, and 15 and 20 μM,
assuming that 15 µM was maximal. Indeed, the life-time was significantly longer at 15 µM (1820 ± 460 ms) compared with 5 µM (1160 ± 150 ms) and 20 µM (1260 ± 280 ms) in all sub-regions of the hippocampus except CA3 stratum oriens and dentate gyrus stratum granulosum. The tendency for oscillations to have long-lasting bursts at intermediate concentrations of carbachol was clear across many amplitude thresholds (Fig. 4.2D) and hippocampal regions (Fig. 4.3A, inset and Supporting Information Fig. 4.S2).

Figure 4.3 Hippocampal oscillations in vitro exhibit a slow decay of temporal correlations, and the oscillation burst life-time has a bell-shape dependence on carbachol concentration. (A) Box plots showing the normalized life-time at the 0.5*median threshold in CA3 stratum radiatum/lacunosum–moleculare (yellow region on the hippocampus diagram). The boxes have lines at the lower quartile, median (red line) and upper quartile values. The whiskers are lines extending from each end of the boxes to show the extent of the rest of the data up to the 1.5 interquartile range of the sample. The hippocampus diagram shows areas with a significant effect of carbachol across the carbachol concentrations of 10, 15, 20 and 25 µM (black dots) (Friedman test, P < 0.05, n > 17, binomial corrected). (B) Box plot showing the DFA exponent fitted in the range 3–20 s for different carbachol concentrations in CA3 stratum radiatum/lacunosum–moleculare. The hippocampus diagram shows the areas with a significant effect of carbachol across the carbachol concentrations of 10, 15, 20 and 25 µM (black dots) (Friedman test, P < 0.05, n = 19, binomial corrected). (C) Box plot showing the dependence of the power on the carbachol concentration in CA3 stratum radiatum/lacunosum–moleculare (median across channels). We did
not observe an effect of carbachol on power across the carbachol concentrations of 10, 15, 20 and 25 μM (Friedman test, not significant, \(n > 9\), binomial corrected). (D) Same as in C, for peak frequency. Black dot: Friedman test, \(P < 0.05\), \(n > 9\), Holm corrected. Note that two recordings were made at 1 μM.

**Hippocampal oscillations exhibit a slow decay of temporal auto-correlations.** The temporal structure of brain oscillations recorded with electroencephalography in vivo shows substantial temporal correlations on time scales up to several tens of seconds (Linkenkaer-Hansen et al., 2001). Whether the temporal structure of oscillations in neuronal networks in vitro also shows temporal auto-correlations with a slow decay is not known. We used the DFA exponent as an index of the correlations from 3 to 20 s (see Materials and methods), and observed a significant variation with carbachol concentration in all sub-regions (0–25 μM, \(n = 18–19\), Friedman test, \(P < 10^{-5}\), Fig. 4.3B). The DFA exponents in CA3 stratum radiatum/lacunosum–moleculare at 15 μM were 0.62 ± 0.03 (\(n = 18\)). These DFA exponents clearly indicate that the correlations were significantly higher than uncorrelated noise (i.e. higher than 0.5, Supporting Information Fig. S5), and they were also higher than those obtained with ACSF (0 μM carbachol) (0.53 ± 0.01; \(P < 0.002\), paired permutation test). We conclude that hippocampal oscillations, even in isolated networks in vitro, can exhibit temporal correlations with a slow decay, and that these are modulated by cholinergic activation. This suggests that the correlations can also be generated in vivo in localized networks without external modulation.

**Carbachol concentration differentially influences peak frequency and power.** The classic oscillation properties of power and frequency may also be sensitive to changing cholinergic drive. The power of oscillations, however, exhibited similar values at carbachol concentrations from 10 to 25 μM (Friedman test; not significant, \(n = 10–14\), Fig. 4.3C). It should be noted, however, that the power is highly variable across slices. In contrast, the peak frequency decreased in stratum radiatum/lacunosum–moleculare of CA3 from 18.0 ± 1.0 to 16.0 ± 1.5 Hz with increasing concentration (\(n = 14\), Fig. 4.3D). Overall, we conclude that the oscillation power does not show the bell-shaped dependence on carbachol concentration in the range from 10 to 25 μM as was observed for the temporal correlations on short and long time scales. However, both life-times and DFA exponents were correlated with power (Supporting Information Fig. 4.53).
Figure 4.4 Prelimbic prefrontal cortex oscillations in vitro exhibit a slow decay of temporal correlations. 

(A) Band-pass-filtered local field potential oscillations (10–25 Hz) from the prelimbic region of a prefrontal cortex slice from a channel with high signal-to-noise ratio (S/N) (top), and from the channel with minimum amplitude (bottom). (B) Oscillations in channels with high S/N have longer life-times. Box plot showing life-times (473 ± 12 ms, $n = 12$) from high S/N channels across slices, and life-times (435 ± 17 ms) from channels with minimum amplitude. (C) Oscillations in channels with high S/N have temporal correlations. Box plot showing DFA exponents (0.58 ± 0.03, $n = 12$) from high S/N channels across slices, and DFA exponents (0.52 ± 0.02) from channels with the minimum amplitude. *$P < 0.05$ (one-tail paired permutation test)

Fast network oscillations in rat prefrontal cortex also exhibit a slow decay of temporal auto-correlations. To investigate whether temporal correlations are a unique phenomenon for mouse hippocampal slices, we analysed the amplitude modulation of carbachol-induced oscillations (25 µM) at 10–25 Hz in rat prefrontal cortex slices. We found that cholinergically-induced oscillations in the prelimbic area of the medial prefrontal cortex have significantly higher DFA exponents (0.58 ± 0.02) and life-times (470 ± 60 ms) in a channel with high signal-to-noise ratio (prelimbic layer 3/5, $n = 10$, Fig. 4.4B and C) compared with the channel with the lowest mean amplitude (0.51 ± 0.02 and 430 ± 20 ms, respectively; $n = 10$) (one-tail paired permutation test), thus showing
that these correlations were not due to correlated background noise in the 10–25 Hz range. The DFA exponents were not different from exponents found for hippocampal oscillations (CA3) at similar carbachol concentrations, but life-times were markedly shorter in the rat prefrontal cortex (Fig. 4.4B and C). We conclude that temporal correlations may be a robust phenomenon of neuronal oscillations in vitro.

Figure 4.5 Temporal correlations are increased by GABAergic potentiation. (A) Box plots of normalized life-times measured using 0.5 median as the threshold. Friedman tests were performed using zolpidem concentrations of 0, 0.1 and 1 μM (all with 5 μM carbachol). Black dots in hippocampus diagrams represent areas with a significant effect of zolpidem application (Friedman test, \( P < 0.05 \), \( n = 11 \), binomial corrected). (B) Same as in A, for DFA. (C) Same as in A, for power. We observed no significant effects on power of zolpidem concentration (Friedman test, \( P > 0.05 \), \( n = 10 \), Holm corrected). (D) Same as in A, for peak frequency. Note that two recordings were made at 1 μM.

Temporal correlations are enhanced by GABAergic potentiation. To investigate whether the temporal correlations could be manipulated, we applied the GABA\(_A\) receptor allosteric modulator zolpidem in concentrations of 0, 0.1 and 1 μM in the presence of 5 μM carbachol. Zolpidem is known to increase the inhibitory postsynaptic current decay time in both pyramidal cells (Goldstein et al., 2002; Cope et al., 2005) and fast-spiking interneurons (Bacci et al., 2003), and has been shown to decrease the frequency of carbachol-induced oscillations in the hippocampus (Palhalmi et al., 2004; Cope et al., 2005; Heistek et al., 2010). We observed that the burst structure of carbachol-induced oscillations was influenced by the potentiation of GABAergic signalling as reflected in
life-times increasing from 1040 ± 1100 to 2450 ± 1910 ms with the application of 1 μM zolpidem in CA3 stratum radiatum/lacunosum-moleculare and stratum pyramidale (n = 11, Friedman test, Fig. 4.5A). We also observed an increase in temporal correlations in these regions, with DFA exponents in CA3 stratum radiatum/lacunosum-moleculare at 1 μm zolpidem of 0.61 ± 0.03 compared with 0.59 ± 0.04 before the zolpidem application, and 0.53 ± 0.02 during ACSF (n = 11, Friedman test, Fig. 4.5B).

Modulation of fast network oscillations with zolpidem at a low concentration of carbachol (5 μM) did not affect power (n = 10, Friedman test, Fig. 4.5C). The peak frequency, however, decreased in all CA3 sub-regions from 18 ± 2 to 14 ± 1 Hz (Fig. 4.5D). We conclude that temporal correlations on both short and long time scales depend on the level of inhibitory activity.

Discussion

Ongoing oscillations in humans exhibit complex fluctuations in amplitude, which are rich in information about the functional state of the underlying networks and thought to play an important role in memory and attention (Raghavachari et al., 2001; Linkenkaer-Hansen et al., 2007; Montez et al., 2009). It is not known, however, whether oscillations in vitro have a similarly rich temporal structure. To test this, we investigated the temporal structure of carbachol-induced oscillations in mouse hippocampal and rat prefrontal cortex slices. We found that the amplitude modulation of mouse hippocampal and rat prefrontal cortex fast network oscillations in vitro exhibits a slow decay of temporal autocorrelations, suggesting that these correlations may emerge due to intrinsic properties of local neuronal circuits. Interestingly, we found maximal life-times and temporal correlations around 10–15 μM carbachol, suggesting that there is an optimal dynamic range of amplitude dynamics around the physiologically relevant levels of cholinergic drive (Menschik and Finkel, 1998). Our results may help to explain why patients with Alzheimer’s disease, known for their cholinergic deficits, have decreasing life-times and temporal correlations as observed for neuronal oscillations in temporo-parietal regions (Montez et al., 2009).

**Life-time and temporal correlation analyses point to an optimal range of cholinergic drive for meta-stable dynamics.** The DFA pointed to a maximum strength of temporal correlations at concentrations of 10–15 μM, and with magnitudes (DFA around 0.6) that were
similar to those observed in human electroencephalography/magnetoencephalography recordings in the beta-frequency band (Linkenkaer-Hansen et al., 2001; Nikulin and Brismar, 2005; Monto et al., 2007). The temporal correlations of fast network oscillations had a different dependence on cholinergic drive compared with the peak frequency and power, which is in line with previous reports showing that temporal correlations are largely independent of the time-averaged power (Linkenkaer-Hansen et al., 2007; Monto et al., 2007). It has been suggested that a slow decay of temporal correlations may arise from sub-cortical modulation or other mechanisms affecting cortical excitability on longer time scales than the duration of individual bursts (Poil et al., 2008). The present findings suggest that temporal correlations can also emerge in local networks without external modulation on long time scales in both the hippocampus and prefrontal cortex. This observation, however, does not exclude a possible additional external influence in vivo. The application of zolpidem led to enhanced temporal correlations, which is in agreement with the effect observed in the beta-frequency band in non-epileptic cortical regions of patients with epilepsy after administration of the benzodiazepine lorazepam (Monto et al., 2007), which is a GABAergic modulator enhancing inhibitory action, as does zolpidem. The life-times of oscillation bursts also increased with the application of zolpidem, suggesting that GABAergic potentiation may stabilize the amplitude of fast network oscillations.

The life-time analysis indicated an increased probability of long-duration bursts of fast network oscillations at intermediate concentrations (10–15 μM) of carbachol. Interestingly, temporal correlations were also maximal at these intermediate levels of carbachol, which are thought to mimic the physiological level of cholinergic input in vivo (Menschik and Finkel, 1998). Thus, the intermediate levels of cholinergic drive provide a maximal dynamic range for meta-stable dynamics. We propose that this maximum may be viewed as a mechanism of stochastic resonance (Gammaitoni et al., 1998), where the cholinergic activation provides just sufficient random excitation (‘noise’) to support the formation of synchronous assemblies, whereas high levels of excitatory drive would disturb the delicate synchrony and stability of the neuronal assemblies.

Interestingly, a maximal dynamic range is increasingly associated with a critical state, which is characterized by balanced network activity and thought to play an important role in the efficient processing of information (Linkenkaer-Hansen et al., 2001; Beggs and Plenz, 2003, 2004; Chialvo, 2004, 2006; Kinouchi and Copelli, 2006; Levina et al., 2007; Beggs, 2008b; Poil et al., 2008; Priesemann et al., 2009). We speculate that temporal correlations at high concentrations of carbachol
decrease because of a changed balance between the excitatory and inhibitory populations (i.e. increased inhibitory dominance due to decreased excitatory to excitatory connectivity and increased inhibitory excitability) (Pitler and Alger, 1992; Hasselmo et al., 1995; Tiesinga et al., 2001) combined with excessively random spiking activity. The increased inhibitory dominance will give rise to oscillations with more uniform amplitudes. The power spectrum analysis may not have captured these subtle differences in oscillatory dynamics, because it only measures the ‘amount’ of activity, but not how this activity is distributed over time (Fig. 4.2F). Overall, our analysis indicates that the amplitude modulation of oscillations can be described as coloured noise or a fractional Brownian process (Touboul and Destexhe, 2010).

A possible link between oscillation life-time, memory and cholinergic deficits in Alzheimer’s disease? A proper level of cholinergic activation is thought to be crucial for learning and memory (Hasselmo, 2006). For example, it is believed that a decreased level of acetylcholine signalling is a key factor causing impaired cognitive function in Alzheimer’s disease (Menschik and Finkel, 1998; Francis et al., 1999; Ikonomovic et al., 2003; Moretti et al., 2004). Interestingly, it was recently reported that the amplitude stability of oscillations is already impaired in the early stages of Alzheimer’s disease (Montez et al., 2009), which is in agreement with the present data showing a high sensitivity of oscillation amplitude stability to changing levels of cholinergic drive. We suggest that biomarkers of amplitude stability based on the analytic techniques used in this study may prove valuable, e.g., in memory-related drug research. For example, future studies should test whether cholinergic or GABAergic manipulations in vivo lead to changes in the amplitude stability of oscillations as observed in the present study, and whether these changes co-vary with performance in cognitive tasks associated with sustained oscillatory activity.

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**Figure 4.S1** The frequency of fast network oscillations depends on temperature. Peak frequency from eight slices from two C57BL6/J mice measured for 10 min for each temperature at 25 µM carbachol. Wash-in at 25 °C for 30 min.
Figure 4.52 Overview of life-time, DFA, power and frequency in different areas and carbachol (carbamylcholine chloride) (CCH) concentrations. Hippocampal diagrams show which area each row represents. Significant changes from 10 to 25 μM CCH are indicated with asterisks. Note that two recordings were made at 1 μM.
Stability of fast network oscillations

**Figure 4.53** Power is correlated with DFA and life-time.  
(A) Power and life-time ranked and correlated. Spearman rho = 0.9, $P < 0.05$.  
(B) Power and DFA ranked and correlated. Spearman rho = 0.7, $P < 0.05$.  
Data in A and B are from a single channel in one slice; sub-region CA3 stratum radiatum/lacunosum–moleculare at 10 μM carbachol.

**Figure 4.54** Parametric statistical tests do not give the same $P$-value as non-parametric tests, if the data do not follow the normal distribution.  
(A) DFA exponents from CA3 stratum radiatum/lacunosum–moleculare. Lilliefors’ composite goodness-of-fit test shows that DFA exponents do not have a normal distribution ($p_L = 0.01$), which is also reflected in the asymmetry of the box plot. The $P$-value from the non-parametric Friedman test ($p_F = 0.06$) is different (and non-significant) from the parametric
two-way anova ($P_a = 0.03$). Note that the non-parametric test is more conservative than the parametric counterpart. (B) Peak frequency from CA3 stratum radiatum/lacunosum-moleculare. Lilliefors’ composite goodness-of-fit test shows that peak frequency values have a normal distribution ($P = 0.2$, i.e. the distributions of data points do not differ significantly from a normal distribution), which is also reflected in the symmetry of the box plots. The $P$-value from the non-parametric Friedman test ($P = 0.001$) is equal to the parametric two-way anova ($P = 0.001$). (C) Histogram showing the non-Gaussian probability distribution of DFA exponents obtained at 10 μM carbachol (see the corresponding box plot enlarged in A). Box interval, 0.05. (D) Histogram showing the Gaussian probability distribution of peak frequency from 10 μM carbachol (see the corresponding box plot enlarged in B). Box interval, 1. Note that two recordings were made at 1 μM.

Figure 4.55 Randomly shuffling the amplitude envelope time series removes the effect of carbachol concentration on DFA exponents. Boxplot of the DFA exponents of signals where the amplitude envelope has been randomly shuffled in mean oscillation cycle sized windows. No significant variation was found between 0 and 25 μM carbachol (Friedman test, $P > 0.05$, uncorrected). Note that two recordings were made at 1 μM.