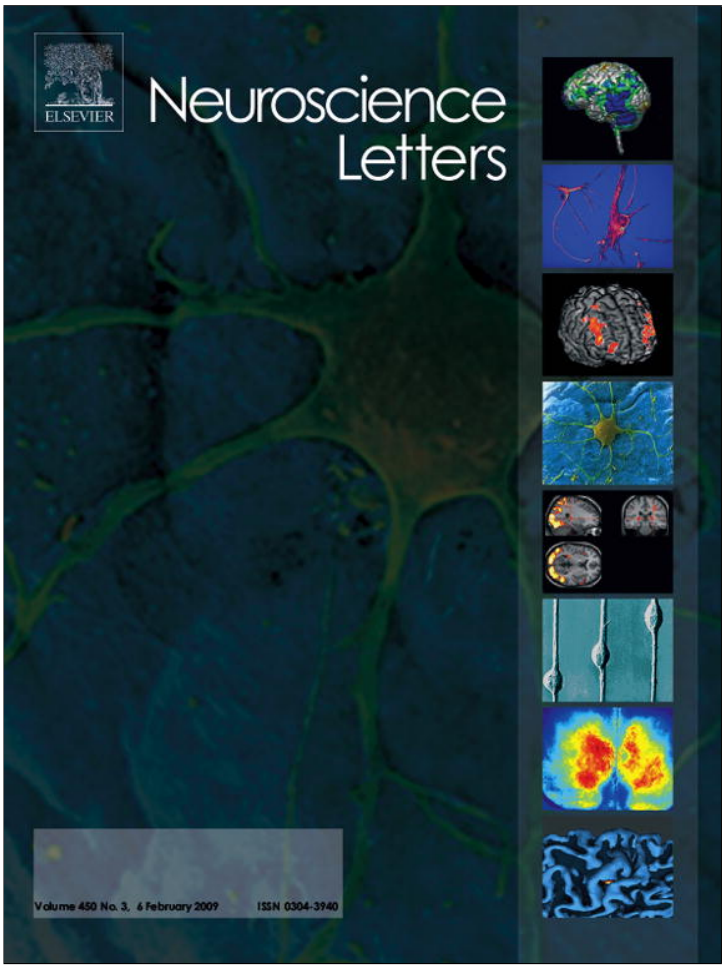

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Measuring sub-millisecond delays in spiking activity with millisecond time-bins

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ABSTRACT

Recent evidence indicates that sub-millisecond delays in neuronal spiking activity may be relevant for neural coding. Estimates of these delays are usually made from cross-correlation histograms (CCH) binned to 1 ms. We investigated the degree to which it is possible to measure delays with sub-millisecond precision when one computes CCHs with bin sizes ≥1 ms. To this end, we introduced sub-millisecond shifts into spike trains recorded from cat visual cortex. The bin sizes of 1/2 to 2 ms were the most optimal for measuring the artificial shifts, even when detecting shifts smaller than 0.5 ms. The results suggest that preferably, one should use CCHs with ~1 ms binning even when investigating differences in delays considerably smaller than 1 ms.

Precise timing of action potentials is important for brain function and has gained growing experimental attention in recent decades. Evidence on the mechanisms of neuronal plasticity [1], input integration [10,18], sound localization [7] and neuronal coding of stimulus properties [14] suggests that the fine temporal structure of neuronal activity in the millisecond, or even sub-millisecond, range may play a significant role in information processing. An important aspect of studies investigating these topics is the precise measurement of delays in neuronal spiking activity [3,8,12,16,17].

Probably the most widely used tool to study temporal relations among neuronal spiking events is the cross-correlation histogram (CCH, Fig. 1A and [4,8,15,16]). A narrow centre-peak in the CCH indicates synchronization between neurons and the shift of the centre-peak away from the midline of the CCH indicates that one neuron tends to fire action potentials before the other (see Fig. 1A). In case of oscillatory responses, these delays in firing are also known as phase offsets and usually do not exceed 10 ms [15,17]. In the past, these delays have been measured by fitting first various functions to CCHs, e.g. cosine or Gabor [9,16], and then determining the point at which the function reaches its maximum.

When computing CCHs, it is necessary to discretize the time delays between action potentials into bins in order to count coincident events across spike trains. In studies of neuronal synchrony, these bins are usually 1 ms wide [4,9]. In contrast, recent studies have indicated that delays could be measured with sub-millisecond precision. Most interestingly, these results were reported not only for high precision time stamps (1/32 ms [17]) but also for spike trains previously binned to 1 ms [12]. This raises the question whether the binning procedure reduces the accuracy with which spiking delays can be estimated, and whether it is possible to estimate these delays with a precision below the resolution imposed by the bin size. One theoretical analysis suggested that for a range up to 2 ms, an increase in bin size should not affect the measurement precision of delays [17]. The explanation is that large bins reduce the error with which the coincidence counts are estimated, providing smooth CCHs and thereby enabling accurate function fitting. As a consequence, for bin sizes up to 2 ms the overall precision of delay estimates is expected to remain constant. We investigated empirically the precision with which spiking delays in CCHs can be measured across different bin sizes. The central question was whether CCHs with the typical bin size of 1 ms can be used to estimate delays with a sub-millisecond precision. To this end, we recorded neuronal responses from cat visual cortex to introduce artificial phase shifts in the range of ±5 ms in steps of 1/32 ms, and tested whether these delays could be detected by the standard methods [8].

In two adult cats, anesthesia was induced with a combination of ketamine and maintained with a mixture of N_2O, O_2 and

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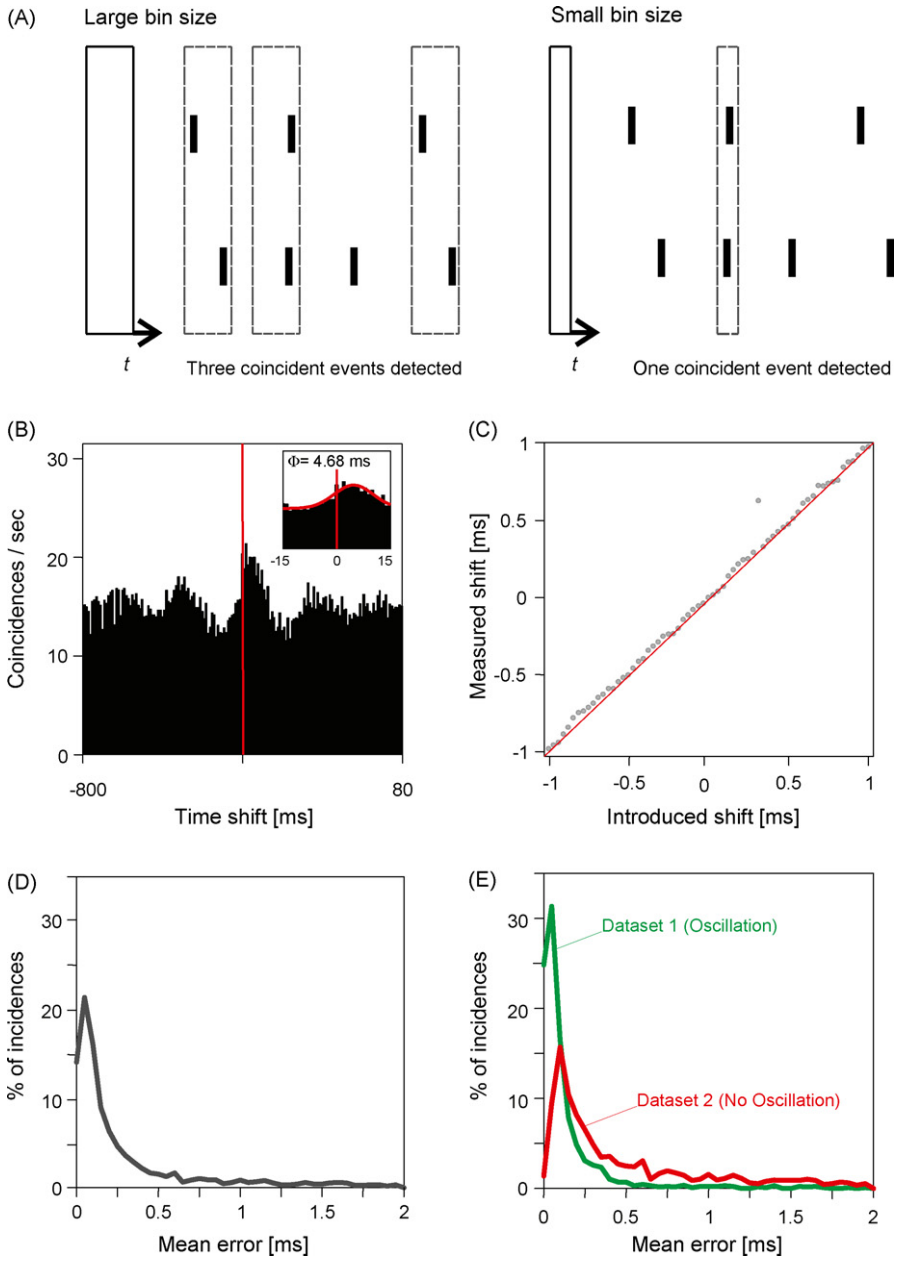

Fig. 1. (A) Schematic description of the procedure for detection of coincidence events based on bins of different sizes. Left panel: a large binning window is slid across a pair of spike trains and three coincident events are detected. Right panel: the same pair of spike trains inspected by a smaller binning window, resulting in detection of only one coincident event. The times of action potentials are indicated by short vertical bars. (B) Example CCH with a large delay, Φ, shown for time shifts ±80 ms. Inset: a zoom into the CCH centre (±15 ms); Red curve: Gauss function fitted to the centre peak. (C) Relation between the amount of delay introduced artificially to the example CCH shown in (B) and delay subsequently estimated by fitting a Gaussian. Red: identity line. (D) Distribution of the mean measurement errors of inserted time shifts across 2880 CCHs for a binning resolution of 1 ms. (E) The same distribution as in (D) but shown separately for the two data sets, one with and one without beta/gamma oscillations in responses. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

halothane. The animals were paralyzed with pancuronium bromide (0.15 mg/(kg h)). All the experiments were conducted according to the guidelines of the Society for Neuroscience and German law for the protection of animals, approved by the local government's ethical committee and overseen by a veterinarian. Further details of surgical and recording procedures are available in [12,16,17].

We recorded multi-unit (MU) activity using Si-based 16-channel Michigan probes. In both cats, the probe was inserted into area 17 of the left cortical hemisphere. The electrode contacts had an impedance of 0.3–0.5 MΩ at 1000 Hz, and were organized in a 4×4 matrix on four shanks, with a distance of 0.2 mm separating the centers of the neighboring contacts. In total, we obtained responses from 32 recording sites in two cats. Signals were amplified first 1000×, then band pass-filtered at 500 Hz to 3.5 kHz, and sampled with a frequency of 32 kHz. Action potentials (spikes) were detected using a two-sided threshold discriminator.

Visual stimuli were presented on a 21-in. CRT monitor positioned at a distance of 57 cm from the eyes. The pupils were dilated with atropine, and the nictitating membrane retracted with neosynephrine. After refraction, the eyes were focused on the monitor by mounting correcting contact lenses. The optical axes of the eyes were aligned by positioning a prism in front of one eye. The receptive fields of all signals recorded within one probe always overlapped, producing clusters spanning up to ~10° of visual angle. The stimuli were positioned such that their centers matched the centre of the RF cluster.

Visual stimuli consisted of high-contrast drifting sinusoidal gratings with a spatial frequency of 2.4°/cycle, covering 12° of visual angle and moving 2°/s, presented binocularly. We varied the gratings' orientation and drifting direction in twelve steps of 30°, covering the entire circle of 360°. Different stimulus directions were presented in randomized order, each stimulus appearing 20 times.

For each recording, we computed CCHs for all possible pairwise combinations of the 16 MU signals recorded simultaneously ($n = 120$). Thus, we investigated the precision of time delays on a total of 240 pairs of units. As we presented twelve stimulus conditions, the study was based on a total of $240 \times 12 = 2880$ pairs of original spike trains, each consisting of 20 stimulus presentations.

For each pair of spike trains, we introduced 321 different artificial shifts by displacing one of the trains in time for an amount that ranged between −5 and +5 ms in the minimal possible steps of the original sampling frequency (1/32 ms). CCHs were always calculated with five different bin sizes: 1/32, 1/2, 1, 2 and 5 ms. The delay of the central peak was estimated for each CCH by fitting the central part, ±15 ms, with a Gauss function and finding the point at which this function reaches its maximum (Fig. 1B). The first 300 ms of responses after stimulus onset were not taken into analysis, CCHs being computed only for the sustained component of the responses in duration of 3.2 s. For the smallest bin size of 1/32 ms, the fit of the Gauss function was poor for ten out of 2880 CCHs (0.3%), for which delays could not be estimated.

We also investigated the robustness of the measurements of delay against a reduction in the amount of data available for the computation of CCHs. To this end, we gradually decreased the amount of data used to compute CCHs, from all 20 trials down to randomly selected 10, 5 or 2 trials.

To estimate the accuracy for measuring artificially induced delays, we calculated the average squared difference between the amount of induced shifts, δ_i ($i \in [1..321]$) and the measured shifts, φ_i. To obtain φ_i, the original offset (i.e., without the artificial shift) was always subtracted from the value obtained from Gauss fit. The error, σ, was then defined as a square root of the average:

$$\sigma = \sqrt{\frac{\sum_{i=1}^{321}(\delta_i - \varphi_i)^2}{321}}, \quad (1)$$

and is expressed in milliseconds.

To assess the strength of beta/gamma oscillations, we analyzed the auto-correlation histograms (ACH; 1 ms binning) by determining the oscillation score (OS) according to [11]. The OS is defined as the ratio between the power at the strongest frequency and the average power of all other frequencies in the band of interest. For the presently investigated frequency range, 20–50 Hz, it is suggested that a response should be considered oscillatory if OS > 5 [11].

To classify the responses of a unit as oscillatory we required that at least three grating directions produced an OS > 5. An entire recording session (i.e., the entire data set) was considered predominantly oscillatory if more than 2/3 of all units were classified as oscillatory.

An illustration of the procedure for the detection of coincident events necessary to compute CCHs is shown in Fig. 1A and the illustration of the procedure for fitting a Gauss function to the centre peak of the CCH in Fig. 1B. We first investigated the precision of estimated time delays from CCHs with bin sizes of 1 ms. In Fig. 1C, we show the relationship between the delays introduced artificially and those extracted by fitting procedures for the example CCH in Fig. 1B. For this case, the shifts could be detected with a precision much higher than the bin size of 1 ms, the average deviation (error, σ) from the true introduced offset being 0.08 ms and correlation coefficient being high, $r = 0.98$. This example was representative of the entire investigated population as the distribution of errors had a median at 0.17 ms (Fig. 1D), the majority of CCHs (61%) having errors <0.20 ms, and only 8% of all CCHs producing errors >1 ms.

The strength of neuronal synchronization depends on the presence of beta/gamma oscillations [2,6]. The strength of synchronization and oscillation may also affect the precision with which the delays are estimated. The two investigated recordings differed in the overall amount of beta/gamma oscillation induced by visual stimulation. While one recording showed strong oscillatory responses with the mean oscillation score (OS) of 3.1 (range 0.24–10.6) (see [1] and methods section for computation of OS), the other recording had mean OS of only 0.5 (range 0.004–3.20). The distributions of errors differed between these two recordings (Fig. 1E), the recording with strong oscillations having higher precision in detecting artificially induced time delays than the recordings without oscillations (median errors: 0.09 ms vs. 0.41 ms). Also, for oscillatory and non-oscillatory responses, the errors stayed below 0.2 ms in 80% vs. 37% of cases and below 1 ms in 98% vs. 84% cases.

We next compared these results to the measurement accuracy of CCHs computed with different bin sizes, by using in total five different values: 1/32, 1/2, 1, 2 and 5 ms (Fig. 2A and B). The errors were lowest for the bin sizes between 1/2 and 2 ms and increased for larger (5 ms) and smaller bin sizes (1/32 ms). This result was the same irrespective of the presence of oscillations. In addition, the recordings with strong oscillations had the advantage in detecting small time delays over the non-oscillatory responses only in the low-error range between 1/2 and 2 ms. Importantly, in most cases the median errors remained below the resolution given by the corresponding bin size for which these errors were computed (indicated by red dashed lines in Fig. 2A and B). As expected, the only exceptions were the errors obtained with 1/32 ms bin sizes.

In Fig. 2C we show how estimates of the delays agreed across CCHs computed with different bin sizes. This analysis was made only for real delays and thus, did not involve insertion of artificial ones. The results are shown for all ten pair-wise comparisons across the five different bin sizes, each histogram showing the entire distribution of differences for all 1440 pairs of spike trains for one recording and for one pair of bin sizes. The results were consistent with the above analysis of artificially inserted delays: The estimates of delays were consistent across bin sizes 1/2, 1 and 2 ms, while the estimates obtained with 1/32 and 5 ms binning deviated from all the others, as indicated by the right-skew of the corresponding distributions. The largest discrepancies were observed for 1/32 ms binning, for which the median difference with other bin sizes ranged between 1.80 and 2.11 ms.

Finally, we tested how the errors of the phase-offset measurements depended on a reduction of the amount of available data. To this end, we computed CCHs using 20, 10, 5 or 2 trials per stimulus condition. Fig. 2D (oscillatory) and E (non-oscillatory recording) show how the median errors increased as the amount of data decreased. With 10 trials, measurement errors increased only marginally, but with further reductions in the amount of data the errors increased rapidly. In the oscillatory data set, this increase in error was fastest for the bin sizes 1/32 and 5 ms, i.e., those that already had largest errors with the full data sets. For example, for the bin size of 1/32 ms the median error increased in total by 1.12 ms (from 2.05 to 3.17 ms), while for the bin size of 1/2 ms the median error increased only by 0.16 ms (from 0.08 to 0.24 ms). In contrast, in the recording lacking oscillations, a reduction in the amount of data produced large increase in errors for all bin sizes and this increase was faster for the three smaller bin sizes of 1/32 to 1 ms than for the larger ones, 2 and 5 ms. In this recording, CCHs computed with 2 ms bins produced most accurate results when the amount of data

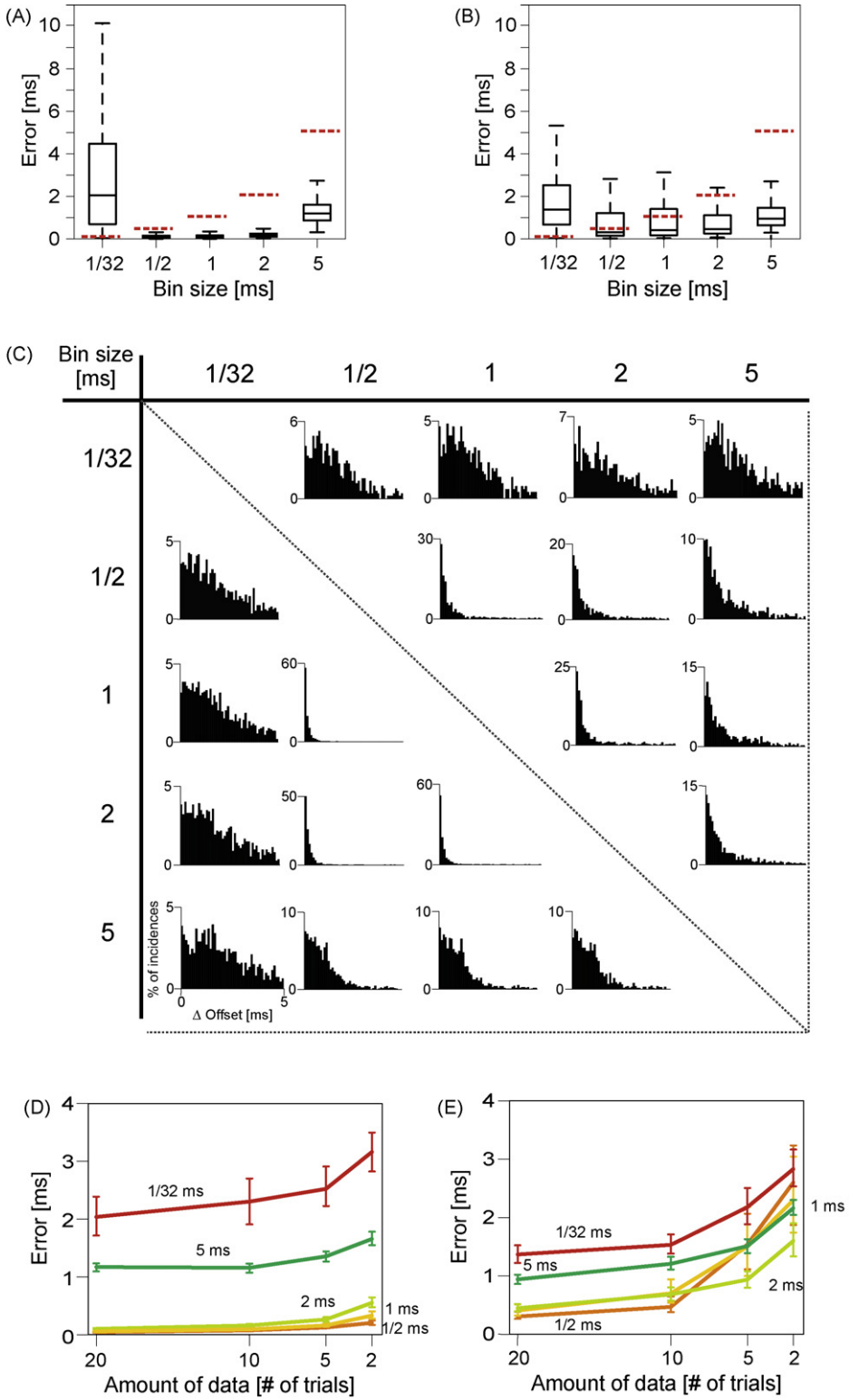

Fig. 2. (A) Distributions of measurement errors for the same errors as in Fig. 1D but obtained from CCHs computed with five different bin sizes in the oscillatory data set. Box-and-whisker plots indicate respectively: Centre line: median; Box edges: 25th and 75th percentile of the error distribution; Whiskers: 1st and 99th percentile. Red dashed lines: the sizes of CCH bins, indicated by the positions on the scale of the error distributions in order to allow direct comparison. (B) Same plot as in (A) but for the non-oscillatory data set. (C) Distributions of the mismatches between the delay estimates with CCHs of different bin sizes. The matrix shows all possible pair-wise comparisons for five different bin sizes. Upper-right triangle: non-oscillatory data set. Lower-left triangle: oscillatory data set. All distributions have the same scale on the *x*-axis covering the range 0–5 ms. (D) Median measurement errors of delays in the oscillatory data set, plotted as a function of the amount of data used to compute CCHs. Different lines represent errors produced with different bin sizes. Error bars: 45th and 55th percentile of the distributions. (E) Same plot as in (D) but for the non-oscillatory data set. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

was reduced. For details on how the number of spikes in the constituting spike trains influences the measurement accuracy of phase offsets, see Supplementary Materials.

The present results suggest that, by computing CCHs, spiking delays can be measured accurately and reliably. Even with binning of 1 ms or larger, CCHs can be used to detect millisecond and sub-millisecond delays in neuronal synchronization. This enables investigation of the spatio-temporal patterns defined by such time delays across populations of synchronized neurons [12,16]. As with stronger oscillations larger fraction of action potentials forms the CCH centre peak, i.e., synchronization is stronger [2,6], we find that the precision of delay estimates also increases.

Interestingly, the bin size with the highest resolution (1/32 ms) yielded the least accurate delay estimates in all analyses. This is a case of a discrepancy between empirical results and a statistical theory stating that, for small bin sizes, binning resolution should not affect the precision of the estimates [17]. Thus, the present empirical results indicate that binning has advantages that were not predicted theoretically, suggesting that some of the assumptions from [17], e.g. error independence need adjustment.

There is increasing evidence suggesting a functional role of the timing of action potentials relative to the endogenously generated rhythms, both in theta [5,13] and in beta/gamma rhythms [3,12,16]. As our results indicate, the CCH is an efficient tool for exploring the underlying temporal relations.

Sub-millisecond delays in neuronal synchronization can be measured from cross-correlograms most optimally with bin sizes 1 or 2 ms. Measurements with small bin sizes, in the sub-millisecond range (1/32 ms) are less precise. In addition to the bin size, the most important factor for a precise measurement of sub-millisecond delays is the presences of beta/gamma oscillations in responses.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neulet.2008.11.066.

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