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# Validation of task-related excess of spike coincidences based on NeuroXidence

Gordon Pipa<sup>a,\*</sup>, Alexa Riehle<sup>b</sup>, Sonja Grün<sup>c,d</sup><sup>a</sup>Frankfurt Institute for Advanced Studies & MPI for Brain Research, Max-von-Laue-Str. 1, 60438 Frankfurt/Main, Germany<sup>b</sup>Mediterranean Inst Cognitive Neuroscience, CNRS & Aix-Marseille Universities, Marseille, France<sup>c</sup>Computational Neuroscience Group, RIKEN Brain Science Institute, Wako City, Japan<sup>d</sup>Bernstein Center for Computational Neuroscience, Berlin, Germany

## Abstract

One of the key findings supporting the assembly hypothesis was found in recordings from the primary motor cortex of behaving monkeys involved in a delayed pointing task [A. Riehle, S. Grün, M. Diesmann, A. Aertsen, Spike synchronization and rate modulation differentially involved in motor cortical function, *Science* 278 (1997) 1950–1953]. Based on the unitary event ('UE') method, the authors have shown that excess coincidences between simultaneously recorded neurons occur dynamically at behaviorally relevant points in time. However, sensitivity of the UE method for non-stationarities and regularity of spike trains caused fear that the results presented might be, at least in part, false positives. We reanalyzed the same data with the new non-parametric method NeuroXidence, which is robust against firing-rate modulations, rate changes across trials, regularity or burstiness, as well as low rates. Our results based on NeuroXidence confirm the results presented in Riehle et al. Spike synchronization and rate modulation differentially involved in motor cortical function, *Science* 278 (1997) 1950–1953 and demonstrate behaviorally modulated excesses of coincidences on a time scale of 3–5 ms.

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**Keywords:** Spike synchronization; Statistical significance; Non-parametric; Surrogate data; NeuroXidence; Monkey motor cortex

## 1. Introduction

In recent years, there has been a great deal of controversial discussion about the assembly hypothesis. On the one hand, evidence for fine-temporal structure in spiking activity is accumulating. On the other hand, there are still concerns about the appropriate analysis techniques to demonstrate the existence of precise temporal coordination of spiking activities of neurons and to diminish the fear of false positives due to violations of inherent assumptions of the analysis method [2,5,13]. One of the key findings that supports the assembly hypothesis was found in recordings from the primary motor cortex of behaving monkeys involved in a delayed pointing task [12]. It was shown that excess joint-spike activity of simultaneously recorded neurons occurs dynamically at behavio-

rally relevant points in time. The analysis of the data was based on the unitary event analysis method ('UE' ; [3,4]).

## 2. UE method

The core idea of the UE method is based on a statistical hypothesis test that compares the amount of empirically measured coincidences within simultaneously recorded neurons with the expected number. The latter is estimated based on the null-hypothesis ( $H_0$ ) that assumes that the recorded spike trains within the analysis window can be described by independent and stationary Bernoulli processes [3]. To compare the expected and the empirical numbers by a statistical significance test, the UE method assumes the expected numbers to be Poisson distributed [3]. Thus, a violation of  $H_0$  can have different causes: spikes of the simultaneously recorded neurons are correlated, or individual spike trains violate inherent assumptions of the method. Of course, the latter has to be avoided because this

\*Corresponding author.

E-mail address: [pipa@mpih-frankfurt.mpg.de](mailto:pipa@mpih-frankfurt.mpg.de) (G. Pipa).

1 may lead to false positives and thus, to false interpretation  
 2 of the data. Specifically, features of experimental data, like  
 3 non-stationarity in time or across trials [4,5], latency  
 4 (co-)variation [2,5], and/or deviation from Poissonian  
 5 processes that may be induced by temporal structures  
 6 within individual spike trains ('auto-structure') [1,4,7,8,10],  
 7 may lead to false-positive results.

8 This caused the concern that experimental evidence of  
 9 precise temporal coordination of spiking activities might  
 10 actually be based on false positives. Therefore, we  
 11 developed a number of additional procedures to increase  
 12 the robustness of the UE method by reducing the number  
 13 of assumptions. We introduced the shuffling of spike  
 14 trains, in combination with bootstrapping, to account for  
 15 the auto-structure of spike trains and their consequences  
 16 for the distribution of the expected coincidences used for  
 17 the significance test [10]. The drawback, however, is that  
 18 the shuffling of spike trains assumes rate stationarity across  
 19 trials.

20 To account for non-stationarity across trials, we  
 21 suggested estimating the expected coincidences and their  
 22 distribution by estimating the firing rates on a trial-by-trial  
 23 approach. Numerically this can be accounted for by  
 24 shuffling the individual spike times within each trial and  
 25 analysis window for each neuron [5]. This approach also  
 26 compensates for co-variation of firing rates across trials.  
 27 However, it does not consider the auto-structure of  
 28 individual spike trains, e.g. a tendency for regularity or  
 29 burstiness. As a consequence, both additional procedures  
 30 [5,10] are not fully robust against features of experimental  
 31 spike trains, since they assume either stationary processes  
 32 across trials or do not account for the full auto-structure.  
 33 This motivated the development of a new approach, called  
 34 NeuroXidence [11], that accounts for both features  
 35 simultaneously and is completely non-parametric.

37

### 38 3. NeuroXidence

39 NeuroXidence is, likewise the UE method, based on a  
 40 hypothesis test that compares the expected and the  
 41 empirical numbers of coincidences occurring in simulta-  
 42 neously recorded spike trains [11]. It detects fine-temporal  
 43 cross-structure on a time scale  $\tau_c$  (e.g.  $\tau_c = 5$  ms) that is not  
 44 explained by either the auto-structure of individual spike  
 45 trains or the cross-structure, which may exist on a time  
 46 scale slower than  $\tau_r$  (e.g.  $\tau_r = 15$  ms). NeuroXidence utilizes  
 47 surrogate data to derive the statistical significance of the  
 48 empirical number of joint-spike events. The surrogate data  
 49 sets are obtained from the original spike trains by  
 50 displacing each spike of an individual spike train by the  
 51 same time interval  $\varepsilon_{t,n}$ . The random variable  $\varepsilon_{t,n}$  is drawn  
 52 independently for each neuron ( $n$ ) and for each trial ( $t$ )  
 53 from a uniform distribution between  $-\tau_r/2$  and  $\tau_r/2$ . Thus,  
 54 random displacements  $\varepsilon_{t,n}$  are bounded by a lower and an  
 55 upper limit:

$$p(\varepsilon_{t,n}) = \begin{cases} \frac{1}{\tau_r} & \text{for } -\frac{\tau_r}{2} < \varepsilon_t < \frac{\tau_r}{2}, \\ 0 & \text{else.} \end{cases} \quad (1) \quad 59$$

60 In contrast to the random temporal displacement (or  
 61 'dithering') of each individual spike [6], which applies  
 62 changes to the original temporal structure of the spike  
 63 trains, NeuroXidence preserves the original auto-structure.  
 64 Thus, fine-temporal cross-structure on a time scale  $\tau_c$  is  
 65 mainly destroyed in the surrogate data, while cross-  
 66 structure slower than the time scale  $\tau_r$  (e.g. rate or latency  
 67 covariation), as well as the full auto-structure of each  
 68 individual spike train, is preserved. To assess a deficiency  
 69 or an excess in the frequency of coincidences for a  
 70 particular set of neurons, we compare the frequency of  
 71 their occurrence in the original data set  $f_t(org)$  to their  
 72 frequency in the surrogate data set  $f_t(sur)$  by computing  
 73 the difference  $\Delta f_t$ .  $\Delta f_t$  is determined for each trial to assess  
 74 its variability across trials:

$$\Delta f_t = f_t(org) - f_t(sur) \text{ for trial } t = 1 \dots T \text{ and} \quad 77$$

$$\bar{\Delta f}_t = \frac{1}{S} \sum_{s=1}^S \Delta f_{t,s}. \quad (2) \quad 79$$

80 To improve the sampling of the chance frequency  
 81 occurring in surrogate data that reflects  $H_0$ , NeuroXidence  
 82 allows the use of multiple ( $S$ ) surrogates. Thus, for  $S > 1$ ,  
 83 the difference  $\Delta f_t$  becomes the average difference between  
 84 the original data and the  $S$  surrogates  $\bar{\Delta f}_t$ . A value of  $\bar{\Delta f}_t$   
 85 larger than zero indicates an excess of coincidences in the  
 86 original data set. In the case of the evaluation of  
 87 coincidences between one pair of neurons, the value of  
 88  $\bar{\Delta f}_t$  is equivalent to the difference of the area under the  
 89 curve of the cross-correlogram between  $-\tau_r/2$  and  $\tau_r/2$  of  
 90 the original spike trains and the more smooth cross-  
 91 correlogram obtained from the surrogate data. To test if  
 92 coincidences are reliably increased across trials, NeuroXi-  
 93 dence tests if the median of  $\bar{\Delta f}_t$ , obtained for all trials  
 94  $t = 1 \dots T$ , is significantly larger than zero (Wilcoxon-test).  
 95 Testing the median rather than the mean or total number  
 96 of JSE across  $M$  trials makes NeuroXidence robust against  
 97 rare events and outliers. This provides, in contrast to the  
 98 UE method, a conservative hypothesis test in the case of  
 99 low rates and non-stationarity across trials.

100 In summary, NeuroXidence allows one to detect an  
 101 excess of precise temporal coordination of spiking activity  
 102 of multiple neurons on a time scale of a few milliseconds.  
 103 Since the estimation of the chance frequency considers the  
 104 complete auto-structure of each individual neuron and trial  
 105 as well as cross-structure slower than  $\tau_r$ , it shows  
 106 robustness against features of neuronal data that were  
 107 discussed to induce false positives [2,5,8], such as any  
 108 firing-rate modulations of individual neurons and covaria-  
 109 tions of firing rates across neurons that are slower than  $\tau_r$ ,  
 110 possible regularity or burstiness, non-stationary rates  
 111 across trials, and low rates. Thus, NeuroXidence provides  
 112 a robust method to test the assembly hypothesis.

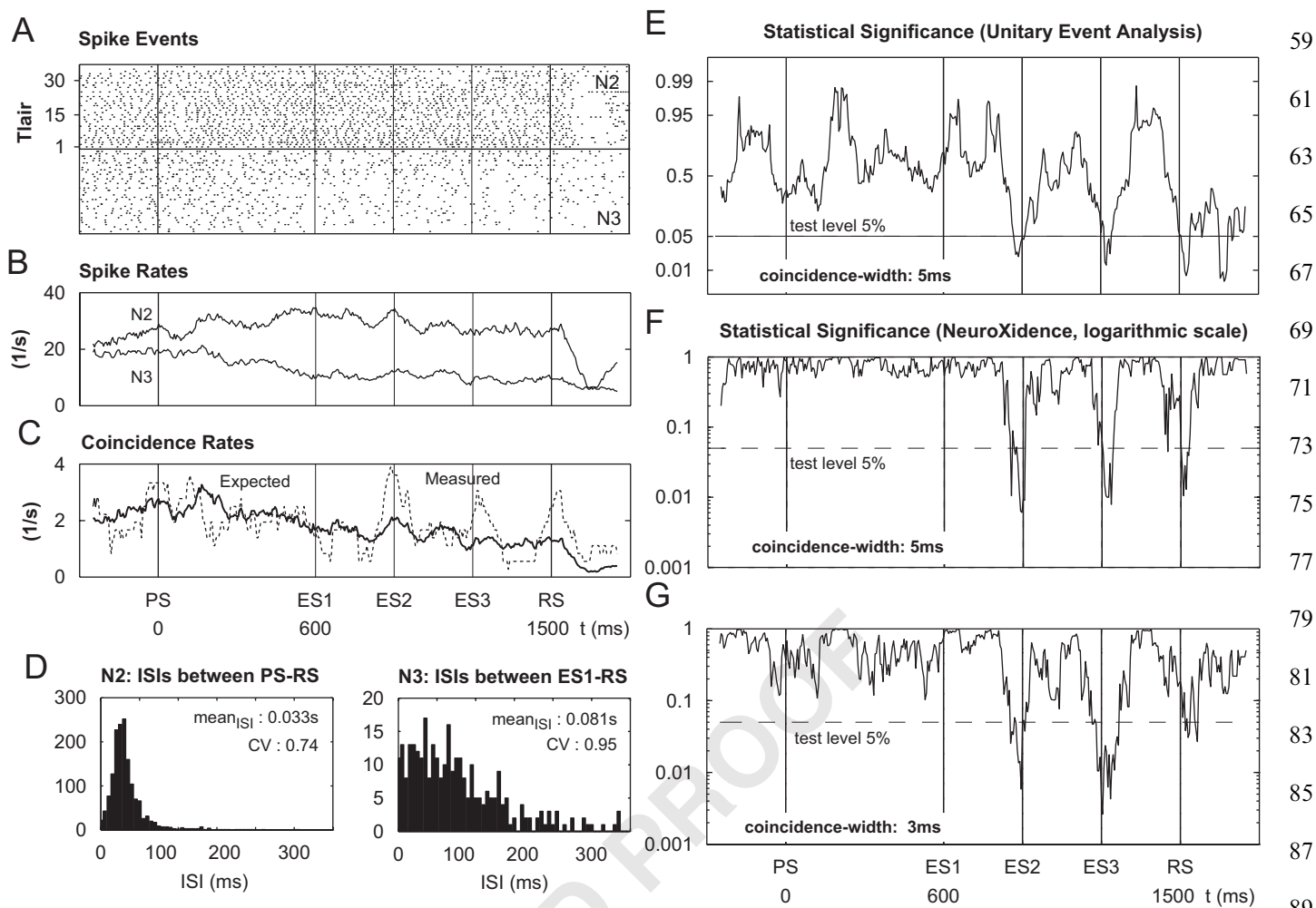


Fig. 1. Spiking activity of two simultaneously recorded single MI neurons in 36 trials (same data as shown in Figure 2 in [12]). Marker on the time-axis in (A,B,C,E,F,G) correspond to the preparatory signal (PS) at 0 ms, the first expected signal ES1 at 600 ms, the second expected signal (ES2) at 900 ms, the third expected signal (ES3) at 1200 ms, and the reaction signal (RS) at 1500 ms. (A) Raster displays of spike discharges (top, neuron 2 ('N2'); bottom, neuron 3 ('N3')). (B) Spike rate for each neuron (computed by a sliding window of 100 ms). (C) UE-based estimation of measured and expected coincidence rates (sliding window of 100 ms shifted by 5 ms, 5 ms coincidence width). (D) ISI histograms for N2 and N3. For N3, five intervals were larger than 300 ms and are not shown in the histogram. (E) Statistical significance for an excess coincidence rate based on the UE analysis (plotted as  $\log_{10}[(1-p)/p]$ , null hypothesis: independent Bernoulli processes). The larger the excess, the lower the  $p$ . (F) NeuroXidence-based estimation of the statistical significance for an excess coincidence rate (null-hypothesis: fine-temporal cross-structure between N2 and N3 is slower than 15 ms, log-scale). (G) The same analysis as in F but coincidence width was 3 ms instead of 5 ms. (E,F,G) Whenever the significance value was smaller than the test level of 5%, the analysis window defines an epoch with significantly more coincidences than expected by chance.

#### 4. Results

We re-analyzed the data presented in [12] with both methods discussed above (UE, [3,4] and NeuroXidence, parameters:  $S = 20$ ,  $\tau_c = 5$  ms,  $\tau_r = 15$  ms). We used the same parameters as those used in the original study (sliding window of 100 ms duration, shifted in 5 ms offset along the data, test level 5%, allowed coincidence width 5 ms). To describe the statistical properties of the recorded spike trains, we computed also the Inter-Spike-Interval ('ISI') distributions and the corresponding coefficient of variations ('CV') for periods with approximately stationary firing rates of the neurons (Fig. 1A–D). The dot-displays as well as the CV of 0.74 of neuron 2 indicate a more regular firing than expected by a Bernoulli process. In a former

study, we found that, for moderate regularity in the spike trains, the significance test that utilizes the Poisson distribution may rather lead to a conservative significance level in the case of the UE method [9]. Only extremely high regularity may lead to false positives [3].

Since NeuroXidence fully accounts for the auto-structure in the data, we also applied that method to the very same data evaluated by the UE method [12] and rigorously compared the results. Fig. 1E,F compare the resulting  $p$ -values of the two methods (log-scale). The exact  $p$ -values resulting from the two methods show deviations, since NeuroXidence tests for an excess of coincidences based on a single-tailed test, while the UE method tests, by definition, on both. However, both methods agree on finding an excess of coincidences, specifically at the very

1 same instances in time, i.e. at times when the monkey  
 2 expected the go-signal to appear (at 0.9, 1.2, and 1.5 s).  
 3 Only during the period after RS do the results of both  
 4 methods disagree. While the UE method indicates a  
 5 significant excess, NeuroXidence indicates chance level.  
 6 The reason may be that neuron 2 changed its activity  
 7 locked to movement onset (not shown here), thereby  
 8 creating a latency variability across trials, when aligned to  
 9 signal occurrence (RS). Realignment of the trials to  
 10 movement onset corrects for that (see [4, Fig. 8]). Another  
 11 cause may be the extremely low firing rate at this time (Fig.  
 12 1A,B), a limitation for the UE method that is discussed in  
 13 [13]. NeuroXidence is unaffected by both influences. Thus,  
 14 the new analysis based on NeuroXidence validates the  
 15 results reported in [12]: significant increase of precise  
 16 coincident spikes beyond chance level is detected by both  
 17 methods at times when the monkey expected the go-signal  
 18 to appear at 0.9, 1.2, and 1.5 s.  
 19 To further investigate the time scale of the underlying  
 20 spike coordination, we shrank the coincidence width from  
 21 5 to 3 ms (Fig. 1G, parameters:  $S = 20$ ,  $\tau_c = 3$  ms,  
 22  $\tau_r = 9$  ms). As with the 5 ms coincidence width, the  
 23 NeuroXidence analysis confirms an excess of coincidences  
 24 with a precision of 3 ms at the same instances in time. We  
 25 conclude that the results shown in [12] indeed indicate an  
 26 excess of synchronized activity on a time scale between 3  
 27 and 5 ms, rather than false positives.

## 29 5. Conclusion

31 Taken together, we conclude that the results shown in  
 32 [12] are not due to an effect of false positives. Indeed, our  
 33 results based on NeuroXidence [11] confirm excess  
 34 synchronized activity on a time scale of 3–5 ms at 0.9,  
 35 1.2, and 1.5 s after PS, at moments in time at which the  
 36 monkey expected the occurrence of the GO signal.  
 37 NeuroXidence does not assume specific properties of  
 38 neuronal data and thus, is robust against features that  
 39 may lead to false positives when using other methods.  
 40 However, it is computationally more demanding e.g.  
 41 compared to the base version of the UE method [3,4].  
 42 Thus, the UE method is a fast and useful tool to screen and  
 43 analyze simultaneous spiking activity for the existence of  
 44 synchronized spiking activity. However, the NeuroXidence

method may be a better selection for validation of the  
 results.

## Acknowledgments

We thank Wolf Singer for stimulating discussions and  
 his support. This work was in part funded by the Hertie  
 Foundation (GP), the Stifterverband für die Deutsche  
 Wissenschaft (SG), and the Volkswagen Foundation (SG,  
 GP).

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