Binary responses are not restricted to anesthetized auditory cortex.

Brief Communication Arising to Nature concerning Wang et al, 2005

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In a recent paper, Wang and colleagues[1] claim that "[Auditory cortical area] A1 neurons [recorded from marmoset monkeys] in the awake condition do not discharge in a binary mode in response to brief tones." Here we argue that (1) the particular analysis they used to draw this conclusion is inappropriate; (2) data published previously by Wang's group actually provide a clear example of binary spiking in A1 of an awake marmoset; and (3) from a computational point of view it is the existence of binary responses, rather than their prevalence, that is of interest.

Since the earliest recordings more than 40 years ago, it has been clear that neurons in the awake auditory cortex show a mix of responses. In ref. 2, for example, 34-36% of neurons in the unanesthetized cat auditory cortex showed transient (phasic) responses, and 28-49% showed sustained responses. However, in the intervening decades attention shifted to the anesthetized preparation, where neurons show mainly transient responses. The last decade has seen a resurgence of interest in the sustained responses more easily studied in the awake animal, and Wang's group has been on the vanguard of this second wave[3].

Recently we reported[4] that a subset (about 50%) of transient responses in the auditory cortex of anesthetized rats showed surprisingly low trial-to-trial variability—0 or 1 spike per trial. We used the term "binary" to describe these reliable responses, in order to distinguish them from the more variable transient "Poisson" responses that have previously been described. Poisson responses have typically been supposed—albeit mainly from visual cortex data—to be a universal characteristic of cortical activity[5,6]. As used in ref. 4, "binary" refers to the (trial-to-trial) variability of a response, whereas "transient" describes its duration; a binary response is necessarily transient, but a transient response need not be binary. Note that we classify responses, not neurons, as binary or Poisson; the same neuron can generate binary responses to one stimulus and non-binary responses to another[4].

The binary nature of a response ensemble is easily obscured when the data set is contaminated by even a small number of non-binary responses. This contamination can arise during data collection, for example through multi-unit recording or imperfect single unit isolation. Fortunately, Wang and colleagues used high-impedance tungsten electrodes to achieve excellent single unit isolation. Unfortunately, in their population analysis of binarity (ref. 1, Fig. 4a), Wang and colleagues explicitly introduce a different form of "contamination" during data analysis by inappropriately pooling responses across *all* neurons, rather than testing for binarity on a response-by-response basis as in ref. 4. Such pooled data cannot be used to test for binarity, since even a small subset of non-binary responses renders the entire ensemble non-binary. In fact, when data from the original binary data set[4] are pooled as in Wang's analysis, the resulting ensemble is also inconsistent with a binary process (see Supplementary Information), contradicting their claim that "[t]he distribution shown in Fig. 4a differs qualitatively from the similar measure obtained in anaesthetized rats[4]."

A key issue therefore is whether binary spiking is merely an artifact of anesthesia, or whether at least some transient responses are binary even in the awake preparation. Fortunately, data from a previous paper by Wang's group (ref. 3, Fig. 4a) depicting activity from a single unit in A1 of an awake marmoset resolves this issue. Careful inspection (*Supplementary Fig. 1*) shows that responses consist of exactly 0 or 1—but never more than 1—spikes on each trial; the probability that such low variability could arise by chance from a Poisson process is $P < 10^{-6}$, and so this response set easily passes the most rigorous test of binarity used in ref. 4 ($P < 0.001$). These data clearly demonstrate that binary spiking is not an artifact of anesthesia, and that it can be readily observed in the awake preparation with high-impedance tungsten electrodes.

It is not yet clear what fraction of neurons in the awake animal are capable of generating binary responses. It may be possible to use online optimization to find stimuli that elicit reliable (binary) responses, just as Wang's group has used online search to find stimuli that elicit sustained responses. Indeed, the same neuron may generate binary responses for some stimuli and sustained responses for others. However, from a computational point of view the significance of binary spiking rests not in its prevalence but rather in its very existence, since it challenges models of cortical function that assume that cortical variability is necessarily high.

In summary, the data in ref. 1 extend previous findings that there exist stimuli capable of driving neurons in auditory cortex to fire in a sustained fashion. However, these data do not support the claim that binary spiking does not also occur in some neurons in response to some stimuli; and the data in ref. 3 indicate that in fact binary spiking does occur in the auditor cortex of awake animals. It remains an interesting challenge to identify precisely the stimulus and behavioral conditions that cause neurons in auditory cortex to fire binary responses.

Supplementary Information accompanies this paper.

Competing interests statement The authors declare that they have no competing financial interests.

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Supplementary Information

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In a recent paper, Wang and colleagues (Wang, Lu et al. 2005) claim that “[Auditory cortical area] A1 neurons [recorded from marmoset monkeys] in the awake condition do not discharge in a binary mode in response to brief tones.” Here we show that (1) data published previously by Wang's group actually provide a clear example of binary spiking in A1 of an awake marmoset; and (2) the particular analysis they used to assess binary firing across their population of recordings is inappropriate, and in fact this analysis yields the same (negative) result when applied to the data set with which we first documented the existence of binary spiking in auditory cortex (DeWeese, Wehr et al. 2003).

The left panel of Fig. 1 reproduces a set of spike rasters from the primary auditory cortex (A1) of an awake marmoset monkey, first published in Fig. 4a of (Barbour and Wang 2003). The right panel shows an expanded view of the responses to several 100 msec duration tones (*green box*) that elicited spikes on some trials. It is evident upon inspection of this figure that all of these responses consist of either one or zero spikes on every trial, with no double or triple spike responses on any trial; we have confirmed with the authors that this is not a printing artifact, and that it does reflect the actual trial-to-trial spike count that they measured (Barbour, personal communication).

We refer to a set of responses such as this one, which consists of only one or zero spikes on every trial, as binary. A cortical neuron that is capable of generating binary responses to repeated presentations of the same stimulus is interesting to us because it is extremely *reliable*—it achieves the lowest possible trial-to-trial variability in spike count given the mean number of spikes per trial (DeWeese, Wehr et al. 2003). We quantified the trial-to-trial variability with the Fano factor (Abbott and Dayan 2001), which is the variance divided by the mean of the spike count across trials. In this example, 37 spikes were observed after 65 trials, so the mean spike count was ~0.57, and the variance was ~0.25, resulting in a Fano factor of ~0.44, which is well below 1—the value we would have expected if the spike counts were drawn from a Poisson process. Since there are no multi-spike events, this is the lowest Fano factor possible for 37 spikes distributed across 65 trials.

Even a highly variable Poisson process can occasionally, by chance, produce a set of responses consisting of only ones and zeros. Therefore, we have applied a procedure we previously developed (DeWeese, Wehr et al. 2003) for determining the statistical significance of apparently binary sets of responses such as this one. Specifically, we computed the cumulative probability for observing a set of responses with a Fano factor that was at least as low as the observed value, under the null hypothesis of a Poisson process fit to the sample mean spike count and the number of trials. Performing this procedure on the data shown in Fig. 1 yields a strikingly low cumulative probability of $P < 10^{-6}$, indicating that it is extremely unlikely that these responses arose due to a statistical fluctuation in an otherwise variable, Poisson-like neuron.

Thus this example easily exceeds the most stringent criterion for binary firing ($P < 0.001$) that we used in our original paper (DeWeese, Wehr et al. 2003). Clearly, binary responses do occur in the auditory cortex of awake animals.

We now turn to our second point, namely that the particular analysis used by Wang and colleagues to assess binary firing across their population of recordings is inappropriate, and in fact this yields the same (negative) result when applied to the data set with which we first documented the existence of binary spiking in auditory cortex (DeWeese, Wehr et al. 2003). Their analysis consisted of generating a histogram of spike counts per trial for each neuron’s best tone. If each and every response were binary, then the histogram would have support at only the 0 and 1 spike bins, but not at 2 or above. However, if even a minority of neurons exhibited non-binary behavior for the best tone, then the multi-spike responses from this minority would “contaminate” the remainder of the binary responses.

We therefore repeated the analysis of Fig. 4a from Wang and colleagues on the data set with which we (DeWeese, Wehr et al. 2003) previously documented the existence of binary spiking. The result is shown in Fig. 2. Despite the fact that over half of the responses in our data set were binary, this pooled analysis produces a histogram that is consistent with a Poisson process (red curve), just as Wang and colleagues reported for their population. This is a simple consequence of the fact that even a small amount of contamination resulting from a few multi-spike trials from a non-binary set of responses (corresponding to a different tone or neuron) can result in Poisson behavior for the pooled data set.

To understand why the pooled analysis of Wang and colleagues failed to reveal the binary nature of our data set, we begin by noting that many neurons exhibited binary responses to some tones and more variable responses to others (DeWeese, Wehr et al. 2003); this is why we were careful to state our results in terms of responses to specific tones, rather than a neuron's pooled responses to many distinct stimuli. In the same way, a neuron might show a sustained response to some stimuli and a transient response to others. Moreover, some neurons in our population did not respond in a binary fashion to any of the tones we presented. Lumping together binary and non-binary responses would therefore not be expected to provide insight. We further note that restricting the analysis to only the best frequency from each neuron is rather arbitrary; across our population, we did not see a systematic increase in the occurrence of binary responses following tones that elicited greater responses.

In summary, the analysis underlying Fig. 4a from (Wang, Lu et al. 2005), which is the basis of their claim that "A1 neurons in the awake condition do not discharge in a binary mode in response to brief tones," cannot be used to support that conclusion.

Wang and colleagues performed a second analysis (Fig. 4b of (Wang, Lu et al. 2005)), which demonstrated that a little over 70% of responses consisted of more than one spike per trial on average. Note that this analysis can effectively distinguish transient from sustained responses, but not binary from non-binary. The result of this analysis is consistent with previous observations that sustained responses in auditory cortex are more common in the awake condition (Evans and Whitfield 1964). We therefore do not expect as large a fraction of responses to be binary in the awake animal as we have found in the anesthetized condition, even if an appropriate analysis were performed to identify binary responses, and even if responses to non-optimal tone frequencies were included in the analysis.

Finally, we observe that if one were to systematically search for stimuli that evoked binary responses from any given neuron, in the same way that Wang and colleagues search for the specific stimuli that elicit sustained responses, it would not be sufficient to test a few examples of non-optimal stimuli, as in Fig. 4 of (Wang, Lu et al. 2005). The space of acoustic stimuli is enormous—even if one is restricted to short tones, one would have to consider a wide range of inter-aural timing and level differences (Brugge, Dubrovsky et al. 1969), for example, which could well play a role in determining whether a response is binary or Poisson. In this regard, it is interesting to note that our recordings were performed with the speaker located to the contralateral side of the animal’s head, whereas Wang and colleagues placed the speaker directly in front of the animal. Discovering the stimulus characteristics that determine whether an auditory cortical neuron responds in a binary or Poisson fashion remains an open, and interesting, question.

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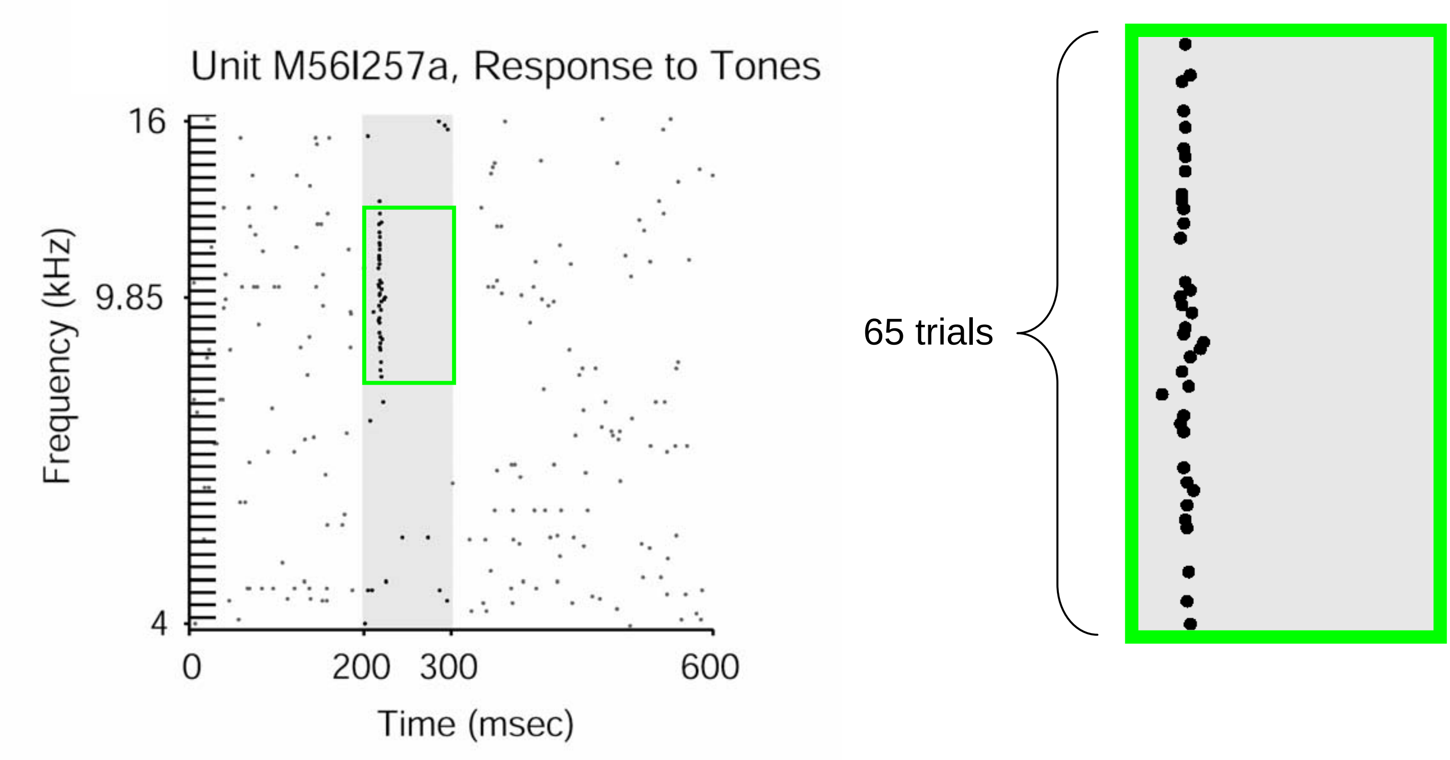
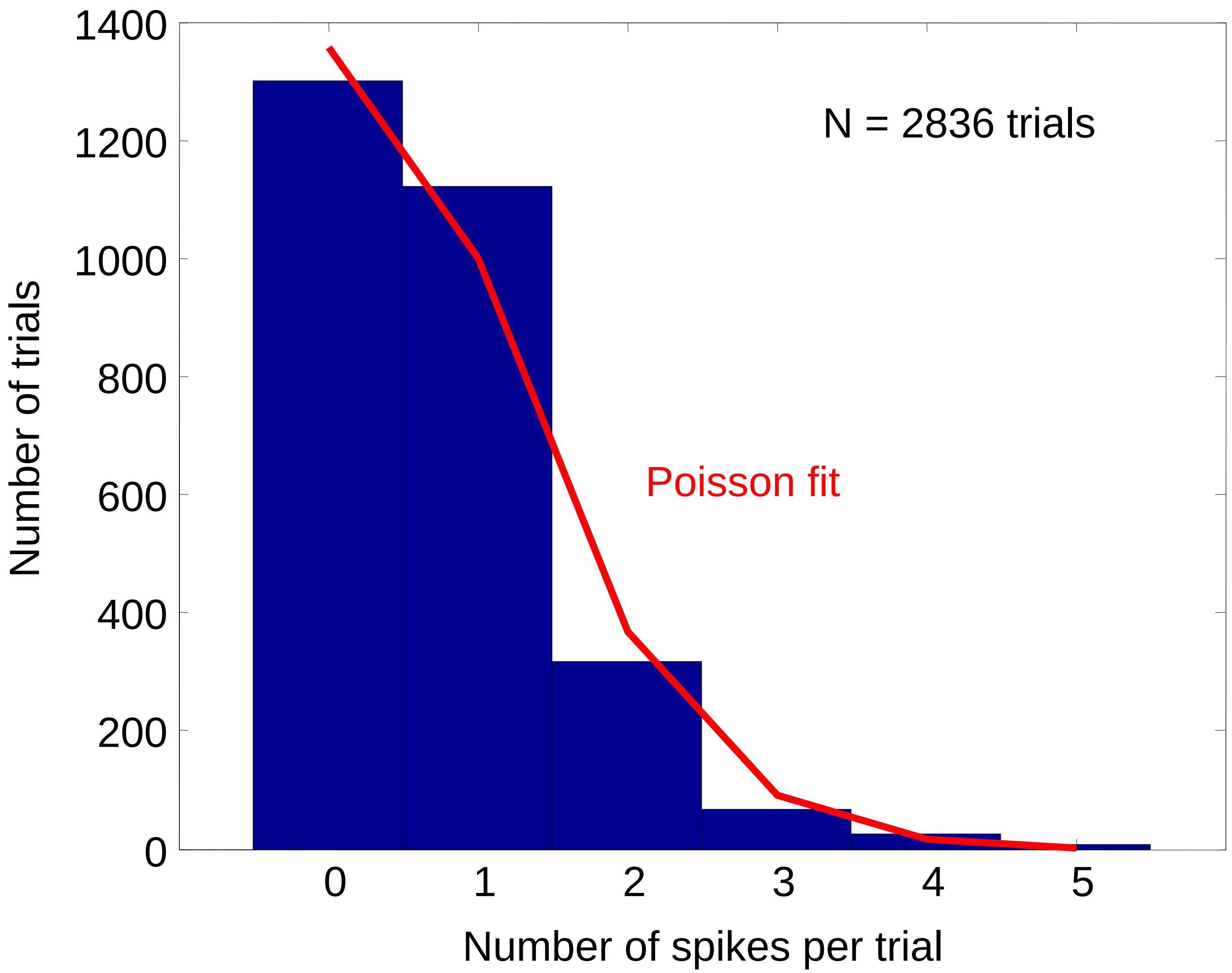


Supplementary Information Figure 1 Neurons in awake auditory cortex can fire in a binary fashion in response to tones. The left panel shows a set of spike rasters recorded from a single neuron in primary auditory cortex of an awake marmoset; this is reprinted from Fig. 4a of (Barbour and Wang 2003). The right panel shows an expanded view of the 65 trials contained within the green box. This set of responses is binary—every trial consists of exactly 1 or 0 spikes. The variability of this set of responses is too low to be consistent with a Poisson process ($P < 10^{-6}$,as quantified by the cumulative probability of the Fano factor; see Supplementary Information text).



Supplementary Information Figure 2 The analysis employed in (Wang, Lu et al. 2005) cannot be used to rule out the existence, or even prevalence, of binary responses. Here we have applied the pooled analysis used in (Wang Lu et al. 2005) to the binary data set studied in (DeWeese, Wehr et al. 2003). The resulting histogram of spikes per trial is well fit by a Poisson distribution, just as it was for the data set from (Wang Lu, et al. 2005).