Reply to Ahissar et al.

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REPLY: We are grateful for the comments by Ahissar et al. on our recent manuscript (Masri et al. 2008) and for the opportunity to respond to these comments. Our manuscript focused on testing the hypothesis—formulated by Ahissar and collaborators—that the paralemniscal pathway in the rat’s vibrissae system functions as a phase-locked loop (PLL) (Ahissar et al. 2000; Sosnik et al. 2001). We emphasize that we do not question the validity of their experimental results or of their analyses. Rather, our goal was to critically test their hypothesis that this sensory system makes use of a PLL mechanism. Two critical predictions emerge from this hypothesis: 1) response latency of neurons in the posteromedial thalamic nucleus (POM) correlates positively with whisking frequency and 2) response duration of these neurons correlates negatively with whisking frequency. In our manuscript we demonstrated that neither of these predictions holds true.

In their Letter to the Editor, Ahissar et al. criticize the experimental approach and the analytical tools we used to disprove the latency prediction; they do not comment on our disproving of the response duration prediction. We believe that their critiques are unfounded.

Simulated whisking

Ahissar et al. attempted to simulate vibrissae movements that occur during “whisking in air”—natural vibrissae movements that occur in the absence of vibrissae contacts with external objects. They do so by applying calibrated air puffs to the vibrissae, to produce vibrissae deflection parameters that replicate kinematics of voluntary whisking. We emphasize that it is highly unlikely that the forces and the resulting transduction events—at the base of the vibrissa follicle—evoked in response to these passive deflections resemble those that occur when the follicle is actively moved by action of the follicular muscles. Rather, these passive deflections mimic contacts of the vibrissae with external objects. Nevertheless, because this was the approach favored by Ahissar and collaborators, we attempted to replicate their paradigm. (We also recorded responses during voluntary whisking in behaving rats; these data are discussed in the following text.)

Central to the Ahissar et al. critique is the argument that the discrepancy between their and our results reflects the use of different parameters of vibrissae stimulation. We agree, and we stated so in our manuscript.

Specifically, Ahissar et al. stress that our stimuli were produced with air pressures that are stronger than those used in their original studies and they question our use of these stimuli. We attempted to precisely replicate their stimulus parameters, although, in our hands, weaker air puffs failed to evoke responses in the majority of POM neurons, rendering it impossible to test the predictions of the PLL model.

However, the absolute value of the air puff pressure is not a reliable metric for comparing stimuli; other factors, such as distance from the vibrissae and the diameter of the air jet are equally important. As Ahissar et al. correctly point out, a more reliable metric is the response profile of neurons in the spinal trigeminal nucleus interpolaris (SpVi), which provides vibrissae inputs to POM.

Ahissar et al. suggest that, as a result of the higher air pressures we used, the response profiles of neurons in the brain stem and thalamus are significantly different from theirs. Specifically, they suggest that our SpVi responses have faster rising edges. Indeed, the three units they show in their Fig. 4B (of Sosnik et al. 2001) appear to have a mean slope of 11.5 spikes/ms (in response to 2-Hz stimuli), whereas the unit we depict in our Fig. 1 had a slope of 15.6 spikes/ms. Therefore we cannot exclude the possibility that subtle differences in stimulus parameters account for the differences between our results. As in our original manuscript, we acknowledge the possibility that the PLL model applies to only a very restricted stimulus parameter space. The main conclusion of our manuscript remains that coding within such a restrictive set of conditions is not compatible with a robust latency coding scheme.

Angular preference

Ahissar et al. also question our decision to test responses of thalamic neurons to the vibrissae deflections at the neurons’ preferred direction. We find this critique puzzling. It is a standard, and an appropriate, practice in physiological analyses to focus on stimuli that optimally drive the recorded neurons. This is particularly important in cases, like the POM thalamus, in which response failures are notoriously high even to optimal stimuli.

Stimulus trains

An important property of POM neurons is their exceedingly high response failure rate; we have argued that this property renders these neurons unlikely to reliably encode vibrissae kinematics, including their frequency. Ahissar et al. suggest that the failure rates we report might reflect our use of long stimulation trains that “are expected to deplete synaptic and cellular resources much more than our brief stimulation trains, particularly at high stimulation frequencies.” That this is clearly not the case is evident from the raster plots we presented (Figs. 2 and 5 in our manuscript), demonstrating that failure rates remain constant throughout the stimulus trains, arguing against the possibility that the responses were “depleted.” Further, at the higher stimulation frequencies (8 and 11 Hz) our stimulus trains were only 6.2 and 4.8 s long, values...
that are not exceedingly longer than those used by Ahissar et al. (3 s). Importantly, we obtained identical results in response to trains of only 10 stimuli (repeated 10 times with 10-s intertrain intervals). Ahissar et al. erroneously assume that these blocks of stimuli were derived “a posteriori” from longer stimulus sequences. This was not the case. In general we find this critique puzzling, in that similarly high failure rates were reported in all previous studies of POm neurons, including those from the Ahissar laboratory (e.g., Fig. 2 in Sosnik et al. 2001).

**Latency analysis**

Ahissar et al. also argue that our respective results reflect significantly different analytical procedures. As clearly indicated in our METHODS section, we applied to all analyses the two latency metrics described in Ahissar’s studies: latency to the first significant bin in the PSTH (T0) and the latency to the half-maximal response (T50). The interpretation of results using either metric is identical: response latency does not correlate with stimulus frequency. Ahissar et al. are correct in pointing out that we did not detail, for every analysis, the numerical results pertaining to each of the two metrics; we felt that doing so would be cumbersome and of marginal utility. Ahissar et al. appear to agree because, in their publications, they commonly detail results for only the T50 metric. Like us, they find that “all these thresholds yielded basically the same results” (Sosnik et al. 2001).

We appreciate the fact that Ahissar et al. took the time to reanalyze and replot our data. However, we find some of their analyses unclear. First, they reanalyzed our data on latency to first spike, pooling data from all neurons and all frequencies. Our approach was only slightly different: we quantified the number of neurons that displayed a significant correlation with stimulus frequency (40.5%), pointing out that this correlation applied only to differences in responses to 2 versus 5 Hz, and then only when using a very liberal latency metric (latency to first poststimulus spike). Because a majority of neurons showed this correlation, it is not surprising that, in the pooled population, there is a correlation between first spike latency (but not any other metric) and stimulus frequency. We stress again that this correlation applied to stimulus frequencies only below or at the low end of normal whisking frequencies (2 and 5 Hz), and using only a liberal definition of response latency. More important, as stated in our manuscript “none of the neurons showed a systematic increase in first spike latency across the four frequencies tested, in contrast to the prediction of the PLL model.”

Ahissar et al. proceed to reanalyze our latency data, making the strong assumption that these data are normally distributed. The reason we did not make this assumption in our analyses is that the data were not normally distributed, which is why we used statistical tests that do not rely on this assumption. Even with an inappropriate use of normal distribution statistics, Ahissar et al. find that our data reveal a correlation between latency and stimulus frequency only in responses to 2 versus 5 Hz.

Ahissar et al. attempt to explain the absence of significant correlations at higher stimulus frequencies by stating that “at 5 and 8 Hz, their latencies approached the maximal possible latency allowed by their paradigm (stimulus duration + input delay ≈ 55 ms).” It is clear from our data that stimulus latencies varied considerably in our population of neurons, with many of them responding at considerably shorter latencies. Significantly, response latency was not predictive of a correlation with stimulus frequency (e.g., Fig. 4 in our manuscript). Thus the lack of correlations at higher stimulus frequencies cannot be attributed to a “ceiling effect.”

**Whisking in air**

Ahissar et al. make the important observation that “latency coding in the POm is sensitive to both stimulus parameters and analysis parameters.” As detailed earlier, we strove to match their parameters in our study. However, this sensitivity significantly limits the robustness of the proposed PLL model. It should also be noted that it is not possible to predict which analysis parameters the brain uses. Therefore we sought to address a key premise of the PLL hypothesis: If POm neurons make use of a latency code, the activity of these neurons must be modulated by whisking in air. That is, their activity must vary with vibrissae movements in air (without contacts).

We reasoned that the most direct and rigorous test would be to analyze responses of POm neurons during natural whisking behavior. However, we found that POm neurons in behaving rats fail to respond during vibrissae movements (Fig. 6 in Masri et al. 2008) and therefore concluded that—at least under our experimental conditions—POm neurons in behaving rats do not encode any feature of whisking kinematics, including whisking frequency. (These findings disprove an additional hypothesis regarding POm function, that is, that they encode sensor motion (Yu et al. 2006).)

We are disappointed that Ahissar and collaborators chose to characterize this part of our study as lacking in rigor. They question our ability to isolate single units and to identify our recording sites as having been in POm; we trust that our previous publications attest to our facilities in these areas; apparently the reviewers of our manuscript agreed. Ahissar et al. also question our decision not to present quantitative data on whisking kinematics. Because our immediate goal was to test the prediction that these kinematics are correlated with neuronal activity, and because we found that none of the POm neurons displayed activity that covaried with whisking, we felt that such an analysis would be immaterial.

Ahissar and collaborators critique our data because of questions related to the “arousal state” of the animals. However, ours was the first study to test responses of POm neurons in awake, freely whisking rats. We believe that data obtained, by ourselves and others, under such conditions are more pertinent than previous data, obtained under deep anesthesia and artificial vibrissae stimulation. Ahissar et al. claim that, by examining our figures, they can conclude that responses recorded in awake rats were of lower magnitude, compared with those recorded under anesthesia. A careful examination of the figures indicates that this is clearly not the case. What is apparent is that the signal-to-noise ratio (that is, the ratio between evoked and spontaneous activity) in our awake rats is smaller than that in anesthetized rats, a finding reported previously for numerous brain regions (e.g., Castro-Alamancos 2004).

**Conclusion**

We welcome the comments by Ahissar and collaborators and the opportunity to continue the discussion on the role of
POm neurons in encoding vibrissae inputs. We carefully considered their critiques and believe that none of them falsifies our original conclusions. Specifically, we stand by our interpretation, based on converging evidence, that the latency of POm neurons cannot consistently and reliably encode whisking or contact frequency. We also stand by our conclusion—unchallenged by Ahissar et al.—that the duration of POm responses also does not correlate with stimulus frequency. Thus the two critical predictions of the PLL model appear to be incompatible with the response properties of POm neurons. Because a previous claim regarding latency codes in the ventroposterior thalamus (Ahissar et al. 1997) was also subsequently challenged (Hartings and Simons 1998), we conclude that PLL-like encoding is unlikely to be a robust feature in the vibrissa-to-cortex pathway.

REFERENCES


