TO THE EDITOR: In a recent Epub issue of the Journal of Neurophysiology, a paper (Masri et al. 2008) appeared in which the authors claim to replicate experiments, but not results, previously obtained in our laboratory (Ahissar et al. 2000; Sosnik et al. 2001). We maintain that Masri et al. 1) did not replicate our experiments, 2) probed the vibrissal system in a different parametric regime, and 3) their results are not inconsistent with ours.

In their paper, Masri et al. set out to test a hypothesis formulated on the basis of experiments conducted in our laboratory—that, in the rat trigeminal pathway, response latencies of neurons of the posteromedial thalamic nucleus (POm) correlate positively with stimulation frequencies. Masri et al. claim to have replicated our experiments using stimuli “quantitatively indistinguishable” from those used in our experiments. Based on the assumption that their experimental conditions accurately reproduced ours, they report that the latency coding reported in Ahissar et al. 2000 and Sosnik et al. 2001 could not be replicated and concluded that “stimulation frequency is not reliably reflected in response latencies of POm neurons.”

However, there are numerous and significant differences in the experimental procedures used by Masri et al. from those described in our papers, the results and conclusions of which they question (Ahissar et al. 2000, 2001; Sosnik et al. 2001). These differences, described in the following text, 1) prevent direct comparisons between the two studies and 2) result in Masri et al. testing the vibrissal system in a parametric regime that might not be representative of its normal working regime.

Whisker stimulation

Stimulus intensity and temporal profile. Masri et al. applied a maximal air pressure of 60 psi, whereas we applied only about 10 psi (0.7 kg/cm²). In our publications, we extensively described and discussed salient differences in responses to fast and slow whisker deflections (see Fig. 1 and the RESULTS and DISCUSSION sections in Sosnik et al. 2001 and the DISCUSSION section in Ahissar et al. 2001). We showed that responses to fast-rising stimuli and to brief stimuli are qualitatively different from those to slow-rising stimuli and to prolonged stimuli. Since natural whisking kinematics are mimicked better by slow stimuli, we consistently avoided using fast stimuli (high psi air puff). Masri et al. do not explain why they chose to use strong (and thus fast-rising) stimuli in their study. Since they do not show the resulting trajectories of whisker motion, their trajectories cannot be directly compared with those we published. However, on close examination of the responses obtained with their stimulation parameters, it is clear that their responses in both the interpolar nucleus of trigeminal complex (SpVi) and POm had faster rising edges than responses we recorded with slow whisker stimulation [compare their Fig. 1 with our Figs. 2, 4, 6, and 7 (Sosnik et al. 2001) for SpVi responses, and their Figs. 2 and 5 with our Figs. 2, 3, 4, 6, and 7 (Sosnik et al. 2001) for POm responses]. This difference in the temporal profile of the response may be crucial for latency coding in the POm. For example, it would be crucial in the case that latency coding results from an interplay between intrathalamic inhibition and the shape of the afferent input to POm, as predicted by Golomb et al. (2006).

Stimulus direction. In Masri et al. (p. 8, lines 2 and 3), whiskers were deflected “in their preferred direction, i.e., the direction that elicited the shortest latency, highest magnitude response.” This is a critical deviation from our protocol, since we always stimulated in the protraction direction, from caudal to rostral. Since rise rates of synaptic inputs and spike thresholds strongly depend on the direction of deflection (Wilent and Contreras 2005), response latencies—and possibly also their frequency dependence—are expected to be significantly affected by differences in the direction of deflection.

The vast majority of neurons in the brain stem nucleus that project to the POm, the rostral part of the SpVi, are best tuned for an upward direction; very few are tuned for a forward direction (Furuta et al. 2006). However, Masri et al. report that in their hands, and using manual classification (how they determined “shortest latency” with manual stimulation is a puzzle), in the POm, “a plurality of our neuronal population has protraction as its preferred direction” (no data were shown). Such transformations between brain stem and thalamus are of course possible. However, if most of their stimulations were in the protraction direction, why didn’t they compare our results specifically with responses in this direction?

Stimulation protocol. In our experiments, we used a block design whereby whisker stimulation was presented during 3-s trials, separated by 2-s intertrial intervals. Such trial durations approximate the typical duration of natural whisking bouts. Although Masri et al. did not include a detailed description of their stimulation protocol, from the text and previous reports it can be deduced that their stimuli were applied throughout a single, long stimulus train. Furthermore, details on train duration and number of deflections applied were also not provided, but once again from the data presented, the long stimulus sequence must have included tens to hundreds of consecutive deflections. (In some RESULTS sections, Masri et al. calculated latencies from “trains of 10 stimuli repeated 10 times.” Since no intertrain intervals were specified, and steady-state re-

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ones were calculated “by eliminating the first 8 trials,” we conjecture that in those cases 10 trains were derived a posteriori from sequential sequences of 100 stimuli, or more, with no intertrain intervals.) Obviously, such long stimulation trains are expected to deplete synaptic and cellular resources much more than our brief stimulation trains, particularly at high stimulation frequencies. Although Masri et al. do not report on the stationarity of responses during their trains, they do state that cells were exhausted (i.e., stopped firing) at stimulation frequencies lower than those in our experiments. Most of the neurons they recorded failed to respond to whisker deflections at rates >5 Hz: 15 of 42 responded at 8 Hz and 7 of 42 at 11 Hz (their Fig. 4) [or, according to the text (p. 10, line 1), only 4 of 42 responded at 11 Hz]. The failure to respond to whisker deflections at frequencies >5 Hz observed by Masri et al. might be due to either the longer stimulation trains or the stronger stimuli (see earlier text) they used. The stronger stimuli may have elicited stronger ventroposterior medial nucleus (VPM) responses and thus a stronger γ-aminobutyric acid type B (GABA_B)-mediated inhibition of the activity of POM neurons (Golomb et al. 2006).

In summation. We stress that we predicted that probing the vibrissal system at a different parametric regime would yield results that differed from ours (Ahissar et al. 2001; p. 364): “Due to the differences mentioned . . . , we would not expect that fast mechanical stimulations of single whiskers would yield results similar to those obtained with our air puffs. We assume that the generation of cortical representations of the whisker frequency depends on the temporal dynamics of the stimulus and on the stimulation field. These dependences have yet to be characterized, by using stimuli, either mechanical or air puffs, with controlled temporal dynamics and stimulation fields.”

Latency analysis

In our papers, we showed that latency coding in the POM is sensitive to both stimulus parameters and analysis parameters. Not only did Masri et al. apply different stimuli, they also analyzed variables different from those we analyzed. We focused on the analysis of latency-to-half-peak (T50) of local populations at steady-state periods, where steady-state periods were defined as 0.5 to 3 s after train onset. Masri et al. focused on the analysis of latency-to-onset (T0) of single units at steady-state periods whose timing varied with the stimulation frequencies (the delay from train onset to steady-state onset varied from 0.7 s at 11 Hz, to 4 s at 2 Hz, and even to 27 s at 0.3 Hz). In some cases Masri et al. did analyze the T50 latency and local populations, but they 1) provided few data and no figures related to these analyses and 2) did not report any analysis of T50 of local populations. Since the latency code described in our papers is described primarily for T50 of local populations, it is puzzling how Masri et al. can claim to have replicated our experimental procedures without analyzing the most significant variable we reported. Even more puzzling is the discrepancy between the data presented by Masri et al. in their figures and their descriptions in the text. Although their data show clear latency coding in specific frequency ranges, they mention nothing about this in their text. Moreover, they describe nonsignificant relations between latency and frequency, even where their data suggest the opposite. We will discuss three such cases: latency-to-first-spike (T1) of single units, T50 of single units, and T0 of local populations.

T1 of single units. The only variable whose entire distribution is presented is T1 of single units (Fig. 4 of Masri et al.). For these data, their null hypothesis—that “response latency was not significantly different across the frequencies tested”—is rejected (P = 0.03, Kruskal–Wallis test, performed in our lab), which was not mentioned in the text. Moreover, there is no mention of the fact that the difference between T1 at 2 and 5 Hz was highly significant (P = 3 × 10−7, paired t-test, performed in our lab).

We compared the distribution of Masri et al.’s T1s with a normal distribution. We found no significant difference: 1) plotting the latencies in a “Normal probability plot” (Matlab) suggested that they were normally distributed and 2) when we simulated 100 normally distributed series with the same sizes, means, and SDs as their T1s in their Fig. 4, none of the simulated populations differed significantly from Masri et al.’s experimental data (P > 0.4, Kruskal–Wallis and P > 0.9, t-test, n = 100 populations).

Since the distributions of Masri et al.’s T50 and T0 were not presented, except for means and SDs, we assume that they were distributed normally as well, and use this assumption in the following statistical calculations.

T50 of single units. Masri et al. (p. 10, lines 18 and 19) state that “This analysis also revealed no statistically significant differences in latencies of responses to different frequencies of stimuli (P = 0.9; in ms: 2 Hz = 25 ± 12, 5 Hz = 40 ± 25, 8 Hz = 33 ± 17, n = 42).” In contrast, an ANOVA test we performed based on these data reveals a significant dependence of T50 on frequency (P = 0.025). Thus either the distribution of T50 of their single units was extremely peculiar or they erred in their calculations of significance. In any case, the difference more important to our discussion is the one between 2 and 5 Hz. When normal distributions of T50 are assumed, latencies to 5 Hz were significantly longer than those to 2 Hz (P = 0.0005, one-sided t-test; performed in our lab).

T0 of local populations. Masri et al. (p. 11, lines 2–4) state that “Similar to single-units, in these local populations there...
was no significant relationship between response latency and stimulus frequency ($P = 0.49$, in ms: 2 Hz = 35.4 ± 4.4; 5 Hz = 47.6 ± 11.5; 8 Hz = 49.0 ± 22.3) ($n = 11$). Again, our ANOVA yields a lower value in this case ($P = 0.07$) and, despite the small sample size, the difference between the latencies to 2- and 5-Hz stimulation was highly significant ($P = 0.002$, one-sided $t$-test).

Thus the main point of Masri et al.—that stimulus frequency does not affect response latency—requires serious reevaluation. What seems to be the case is the following. Under the conditions tested by Masri et al. the vibrissal system seemed to be pushed to a working regime with extremely long latencies [compare their latencies (gray) with ours (black) in Fig. 1 here]. In fact, at 5 and 8 Hz, their latencies approached the maximal possible latency allowed by their paradigm (stimulus duration + input delay $≈ 55$ ms). This can explain the lack of significant differences between latencies at 5 and 8 Hz and the high failure rate at $≥ 8$ Hz, since the probability that a neuron in the sensory pathway will start responding after its afferent input is removed is very low. Thus due to this ceiling effect, the only comparison that appears to be valid in Masri et al.’s data is that between 2 and 5 Hz and, in this range, latency coding appears to be highly significant. Interestingly, their latency-to-frequency slope in the 2- to 5-Hz range is similar to the slope in the 5- to 8-Hz range in our study (Sosnik et al. 2001) (see Fig. 1 here).

This suggests that the vibrissal system exhibits latency coding in different frequency ranges under different stimulation regimes. Whether a phase-locked-loop-like (Ahissar 1998), intrathalamic GABAergic (Golomb et al. 2006), or a different mechanism underlies this persistent latency coding in PoM is not yet known. In any case, each of these mechanisms will function differently in different ranges of stimulus parameters, primarily of stimulus frequency and velocity. We consider variations in the extent of coding under various conditions as potential indicators for the actual mechanisms underlying latency coding in this system, and for their working ranges.

**POm responses in the awake rat**

Data from experiments in awake rats were used by Masri et al. to extend their latency measurements and to “directly test” our hypothesis that PoM neurons preferentially respond to whisking movements (Yu et al. 2006). Disappointingly, the method and quality of their presentations preclude a serious analysis that is at least as comprehensive as that of the original work. Unfortunately, Masri et al. provided a partial and incomplete study, whose stated conclusions cannot be justified. Although their paper focuses on challenging our published papers and hypotheses, they do not appropriately address the validity and interpretations of our data presented therein. Their experimental conditions are far from being “quantitatively indistinguishable” from ours. Furthermore, where comparisons were made, the analysis of important aspects differed. These differences prevent a direct and valid comparison with our results. Nevertheless, the data of Masri et al. can be considered as an extension to our study. The implication, from their data—that in different parametric regimes the PoM exhibits latency coding at different frequency ranges (Fig. 1 here)—suggests that latency coding is a robust feature of PoM.

**Concluding remarks**

Although the attempt of Masri et al. to replicate our original findings is welcome and timely, they do not follow traditional norms for challenging the validity of earlier works by providing a comprehensive analysis that is at least as comprehensive as that of the original work. Unfortunately, Masri et al. provided a partial and incomplete study, whose stated conclusions cannot be justified. Although their paper focuses on challenging our published papers and hypotheses, they do not appropriately address the validity and interpretations of our data presented therein. Their experimental conditions are far from being “quantitatively indistinguishable” from ours. Furthermore, where comparisons were made, the analysis of important aspects differed. These differences prevent a direct and valid comparison with our results. Nevertheless, the data of Masri et al. can be considered as an extension to our study. The implication, from their data—that in different parametric regimes the PoM exhibits latency coding at different frequency ranges (Fig. 1 here)—suggests that latency coding is a robust feature of PoM.

**Acknowledgments**

We thank P. M. Knutsen, I. Saraf-Sinik, K. Bagdasarian, and B. Schick for reviewing this letter and G. Horev for performing statistical analysis.

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