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Hierarchical unimodal processing within the primary somatosensory cortex during a bimodal detection task

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Do sensory cortices process more than one sensory modality? To answer these questions, scientists have generated a wide variety of studies at distinct space-time scales in different animal models, and often shown contradictory conclusions. Some conclude that this process occurs in early sensory cortices, but others that this occurs in areas central to sensory cortices. Here, we sought to determine whether sensory neurons process and encode physical stimulus properties of different modalities (tactile and acoustic). For this, we designed a bimodal detection task where the senses of touch and hearing compete from trial to trial. Two Rhesus monkeys performed this novel task, while neural activity was recorded in areas 3b and 1 of the primary somatosensory cortex (S1). We analyzed neurons' coding properties and variability, organizing them by their receptive field's position relative to the stimulation zone. Our results indicate that neurons of areas 3b and 1 are unimodal, encoding only the tactile modality in both the firing rate and variability. Moreover, we found that neurons in area 3b carried more information about the periodic stimulus structure than those in area 1, possessed lower response and coding latencies, and had a lower intrinsic time scale. In sum, these differences reveal a hidden processing-based hierarchy. Finally, using a powerful nonlinear dimensionality reduction algorithm, we show that the activity from areas 3b and 1 can be separated, establishing a clear division in the functionality of these two subareas of S1.

multisensory processing | unimodal coding | cortical hierarchy | bimodal detection task | primary somatosensory cortices

Significance

Our brain integrates information from all our senses to perceive the external world. But where and how in the brain this integration occurs? Here we ask if the primary somatosensory cortex (S1) encodes information from more than one sensory modality. We recorded the activity of single neurons from areas 3b and 1 of S1, while trained monkeys performed a bimodal detection task, where tactile and acoustic stimuli compete. The analysis showed that neurons from areas 3b and 1 responded only to the tactile modality both in their rate and variability. However, our results support that these two areas are different enough as to be considered functionally distinct entities.

Have you ever turned down the volume on your car's radio when looking for a place to park or closed your eyes when trying to detect a sound? Sometimes, the inputs from our different senses can interfere or compete with each other, even though integrating them is essential to form a coherent perception of the external world. Neural correlates of this competition should emerge in areas of the brain that participate in multisensory integration. The search for where and how in the brain this operation is carried out is an active area of research. In particular, the question of whether primary sensory cortices have any explicitly multisensory responses has been the subject of much discussion. The mainstream view suggests that this is not the case; primary sensory cortices are unimodal, processing only their own sensory modality. In this work, we investigate if areas 3b and 1 of the primary somatosensory cortex (S1) show multisensory processing. We do so by analyzing the firing rate coding and variability of single neurons, recorded while Rhesus monkeys performed a bimodal detection task (BDT) that involved competition between the senses of touch and hearing.

It was thought that multisensory processing, at the neural level, came only after intensive unimodal processing (1, 2). Surprisingly, recent decades have brought several studies arguing that it also occurs in areas considered thoroughly unimodal, such as the primary sensory cortices (3–13). This would mean that S1, for example, not only processes tactile but also auditory and even visual information (3, 14). Similarly, the auditory cortex would process tactile and visual stimulus properties in addition to auditory ones. However, this growing body of evidence comes mainly from studies that use recording techniques with low (EEG, fMRI) or medium (LFP) spatial resolution, but even in single-unit recordings (3, 13, 14). Further, their experimental designs have led to inconclusive findings due to lax control of stimulus properties or the simultaneous presentation of complex stimuli from different modalities, usual properties in naturalistic stimuli; still, such an approach could promote cooperative multimodal mechanisms in primary sensory cortices than in reductionist, highly controlled stimuli might fail to elicit. However, this could confound multisensory responses with unimodal ones to uncontrolled properties that are correlated between stimuli of different modalities.

For our study and assuming the unimodal tactile processing for S1, we asked two core questions for the acoustic modality: Is there a change in the firing rate of S1 neurons during an acoustic stimulus? In their variability? Is there any difference in the response

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properties of neurons in 3b vs area 1 during acoustic stimulation? There is, nonetheless, an alternative hypothesis to multisensory processing: attention, which could explain some of the differences in neural responses between unimodal and multimodal tasks. However, previous evidence (15) has shown that performance in detection tasks is not affected by dividing attention between two senses. Consequently, in our present study we focus on bimodal detection. Still, we do comment further on attention in the Discussion section.

This work contributes evidence that could address some of the drawbacks of previous studies. First, electrophysiological data were recorded with high spatial resolution. We obtained spike trains from single neurons recorded using independently moving microelectrodes and a custom offline spike sorting algorithm. Second, a BDT was used, in which interleaved vibrotactile and acoustic stimuli of varying amplitude were delivered. This means that the stimuli presented could be above, below, or at the threshold for detection, putting more emphasis on the responses of S1 (16). Third, we analyzed both the stimulus coding and variability of spike trains. On the one hand, coding could be considered the foremost functional feature of neural responses for cognition, and thus is fundamental for understanding the role of an area in brain processing. On the other, variability is an important metric in which coding is constrained and has rarely been studied in the context of multisensory processing. Finally, we analyze area 3b and 1 separately, and we further separate neurons within each area by the relative position between their receptive field and the stimulation site (center [RF_1], periphery of the center [RF_2] and far away from the center [RF_3]). Separating our data in this way allows us to address a number of issues: a) the functional differences between these two areas, which are often overlooked, but which previous evidence has pointed toward (17), starting with the observation that each one has a complete somatotopic map of the body (18, 19); whether neurons present multisensory responses due to b) their receptive field, c) their area, or d) the presence of functional clusters within each area. All these possibilities are obfuscated in studies with lower spatial resolution but have important implications: if S1 neurons respond to acoustic stimuli, for example, the foundational concept of receptive fields must be reconsidered.

In summary, our work analyzes the firing rate coding and variability to 1) uncover evidence of areas 3b and 1 carrying out multisensory processing, and 2) characterize the differences between responses in these areas. Our analyses were done at both the single neuron and population levels. Regarding the first point, the results that follow establish that these areas only exhibit unimodal responses to the principal modality and not the acoustic stimuli. Multisensory processing is a controversial subject for which some studies (3–13) have found positive results, while others (20, 21) yielded no evidence of such processing in primary sensory cortices. As for the second point, the results that follow establish that these areas exhibit distinct responses, with area 1 showing more variability, integration, and processing. Our study finds that, even though these areas differ in firing rate coding, latency, variability, and intrinsic timescale, both are only modulated by tactile stimuli. This leaves areas 3b and 1, components of S1, as unimodal.

Results

BDT. Two monkeys (*Macaca mulatta*) were trained to perform the BDT, which consisted of reporting the presence or absence of a vibrotactile (Tac) or acoustic (Ac) stimulus, presented in the range from sub- to suprathreshold intensities. Both types of stimuli had a

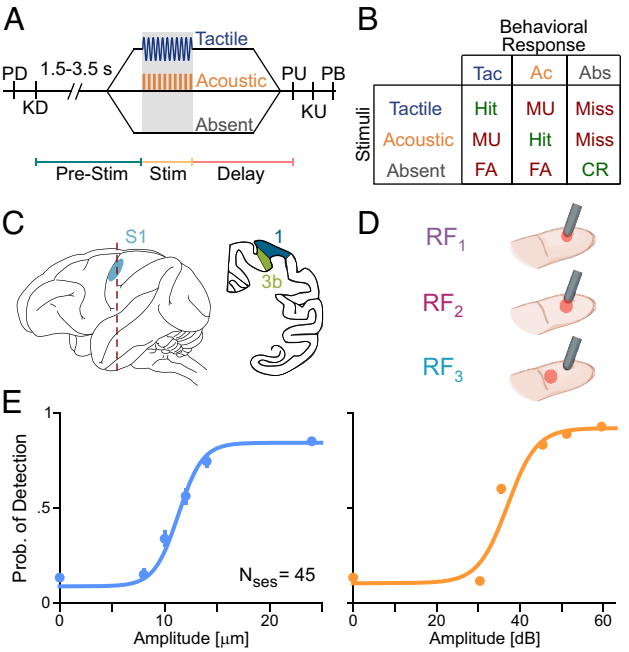

Fig. 1. (*A*) Schematic representation for the BDT. Trials start when a mechanical probe indents the glabrous skin on one fingertip of the monkey's restrained right hand (probe down event, "PD"). Immediately after PD, animals respond by placing their free left hand on an immovable key (key down event, "KD"). After KD, a variable period (1.5 to 3.5 s) is presented to prevent the animal's prediction of stimulus onset. This is followed by a stimulus presentation (0.5 s), which can be tactile, acoustic (both 20 Hz), or stimulus absent. After stimulation, a fixed delay period (2 s) is presented followed by the probe up event ("PU"). This last event serves as the "go" cue for the monkey to release its hand from the key (key up event, "KU") and report its decision using one of the three buttons placed in front of him, at eye level (push button event, "PB"). The three buttons indicate the following responses: "tactile stimulus present," "acoustic stimulus present," and "stimulus absent." Correct responses were rewarded with a few drops of fruit juice. (*B*) Chart of trial outcomes according to stimuli versus behavioral responses. Correct responses are colored green and incorrect responses are colored red. In the absence of stimuli, an incorrect "stimulus present" response indicates a false alarm, while a "stimulus absent" response results in a correct rejection. When a stimulus is present, a hit trial indicates a "present" response matching the given stimulus modality; a trial is misunderstood (MU) when the "present" response does not match the modality; finally, a trial is a miss if an "absent" response is given. (*C*) Illustration of the brain's left hemisphere with S1 marked (*Left*, light blue) and a coronal brain slice (*Right*) with the areas 1 (blue) and 3b (green) highlighted. (*D*) Illustration of finger with receptive field locations (RF_1, RF_2, RF_3) relative to the mechanical probe. (*E*) Psychometric curves for tactile (blue) and acoustic (orange) stimulation. Each modality had six classes of stimuli ranging from 0 to 24 μm or from 0 to 59 dB with a total of 6,750 trials over the course of 45 sessions.

fixed frequency of 20 Hz and lasted 0.5 s. For the tactile condition, amplitude of stimuli delivered to the skin was modulated while for the acoustic condition, the volume of a 1 kHz pure tone was adjusted. Both modalities were interleaved with an equal number of trials where no stimulus was delivered (Abs). Animals pressed one of the three push buttons to report their choice: Tac, Ac, or Abs (Fig. 1 *A* and *B*). Push buttons were located a reaching level in the left quadrant relative to the midline of the animal's body. While monkeys performed the BDT, neuronal activity was recorded within S1 (areas 3b and 1; Fig. 1*C*) and neurons were classified according to the relative position between stimulation zone and receptive field (Fig. 1*D*) (see *SI Appendix* and Fig. 1*D* for RF definitions: RF_1, RF_2, and RF_3). Psychometric measurements for both modalities showed that animals were trained to perform the task up to their psychophysiological thresholds (Fig. 1*E*).

Response Properties of S1 Neurons. We recorded activity from 67 neurons in area 3b and 313 in area 1, while monkeys performed the

BDT. Based on recent evidence that suggests differences in their hierarchy (17), throughout this manuscript we analyzed neurons from areas 3b and 1 separately. For area 3b, we found: 45 neurons (67.2%) that corresponded to RF_1, 10 (14.9%) to RF_2, and 12 (17.9%) to RF_3. For area 1, 81 (25.9%) corresponded to RF_1, 108 (34.5%) to RF_2, and 124 (39.6%) to RF_3. Fig. 2 and *SI Appendix*, Fig. S1 show the responses of three individual neurons recorded in areas 1 (RF_1 and RF_3) and 3b (RF_2) (and vice versa in *SI Appendix*, Fig. S1, where an RF_2 neuron from area 1 and RF_1 and RF_3 neurons for area 3b are displayed) with their corresponding normalized activity, during the tactile and acoustic trials. It is important to note that RF_1 neurons faithfully represent intensity modulations of vibrotactile stimuli (Fig. 2*A* and *SI Appendix*, Fig. S1*A*) while, despite belonging to the same brain area, this property is diluted in RF_2 (Fig. 2*B* and *SI Appendix*, Fig. S1*B*) and RF_3 (Fig. 2*C* and *SI Appendix*, Fig. S1*C*) neurons. Notice that RF_2 neurons responded only during suprathreshold stimulation (dark pink). On the other hand, no activity modulation was found during acoustic stimulation regardless of the RF location or brain area, which suggests that the responses originating from cortical areas 3b and 1 are insensitive to acoustic stimuli, regardless of their stimulus intensity. To test this observation in the full population, we computed the normalized activity of all RF_1 neurons during tactile and acoustic stimulation for both brain subareas (Fig. 3 *A* and *B*). The results showed the same pattern as in the individual units, where neural responses in 3b and 1 were actively modulated in the tactile condition but not in the acoustic one. Similarly, as was observed in RF_2 and RF_3 single neurons, neural population activity diminished in both brain areas for these RFs (*SI Appendix*, Fig. S2 *A* and *B*) without showing any sort of modulation during acoustic stimulation were applied, no matter the intensity (*SI Appendix*, Fig. S2*C*). Again, the RF_2 population modulated its activity only with suprathreshold stimuli. Additionally, it has been reported (22) that multiunit firing rate activity is correlated with the gamma band from the LFP. So, to indirectly test whether there was a multimodal response in the aggregate neural activity, we pooled the spikes recorded simultaneously and calculated the normalized firing rate per amplitude and amplitude mutual information for each modality (*SI Appendix*, Fig S3). However, we found no differences from the results with well-isolated responses. In other words, aggregate neural activity shows no changes during the acoustic stimuli compared with the no stimulation condition, but it does change with tactile stimulation. This could have been expected, since the well-isolated units aggregated for this analysis had been shown to have no acoustic information when taken separately.

These results suggest that neurons in areas 3b and 1 are unimodal. Nevertheless, we still wondered whether there was some slight modulation in the neural activity during the presence of acoustic stimulation. To further investigate such a possibility, we performed neurometric analysis with the neural activity data, using the optimum criterion technique. Given that sensory neurons are recognized for accurately codifying the frequency of stimuli (23, 24), we also computed the neurometric curves using the Fourier transform of the firing rate during the stimulation epoch. *SI Appendix*, Fig. S4 *A* and *B* shows neurometric curves for neurons of areas 3b for the tactile and acoustic experimental conditions. In agreement with previous studies (16, 23), neurons from both areas yielded a strong and marked response when the tactile stimulation set was applied. Further reaffirming this behavioral response, periodicity neurometric curves showed similar results: no modulation for the acoustic stimuli, while in the tactile modality there was a detriment in the periodicity for the neurons from area 1 compared with those in area 3b. These results indicate that neurons from areas 3b and 1 respond to tactile stimuli while remaining unaffected by acoustic stimuli.

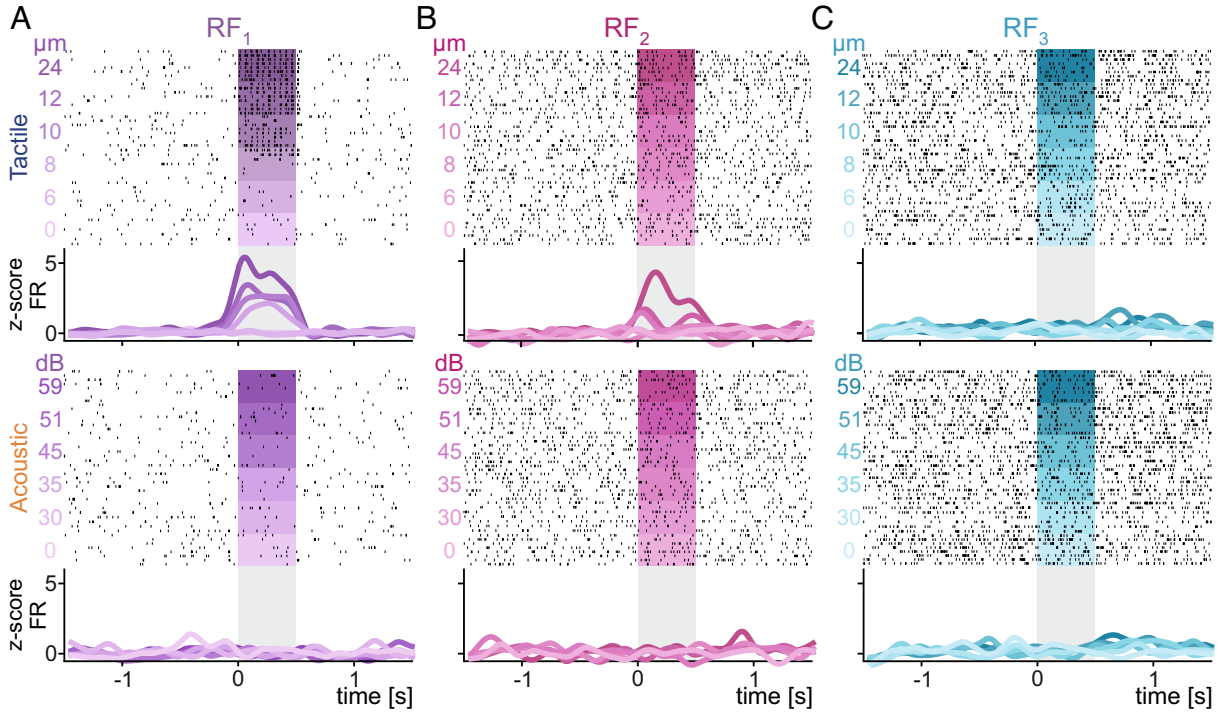

Fig. 2. (*A–C*) Raster plots of three neurons recorded with different receptive fields. Normalized neuronal activity is shown below each raster. Neurons were recorded in area 1 (*A* and *C*) and 3b (*B*), for the three different receptive fields (RF_1, purple; RF_2 pink; RF_3 blue), in the tactile (*Top*) and acoustic (*Bottom*) modalities. In the raster panel, black ticks represent neuronal spikes while colored rectangles represent the stimulation period. On the other hand, in the activity panel, colored lines represent the average of normalized neuronal activity obtained from trials with the same stimulus class and gray rectangles represent the stimulation period.

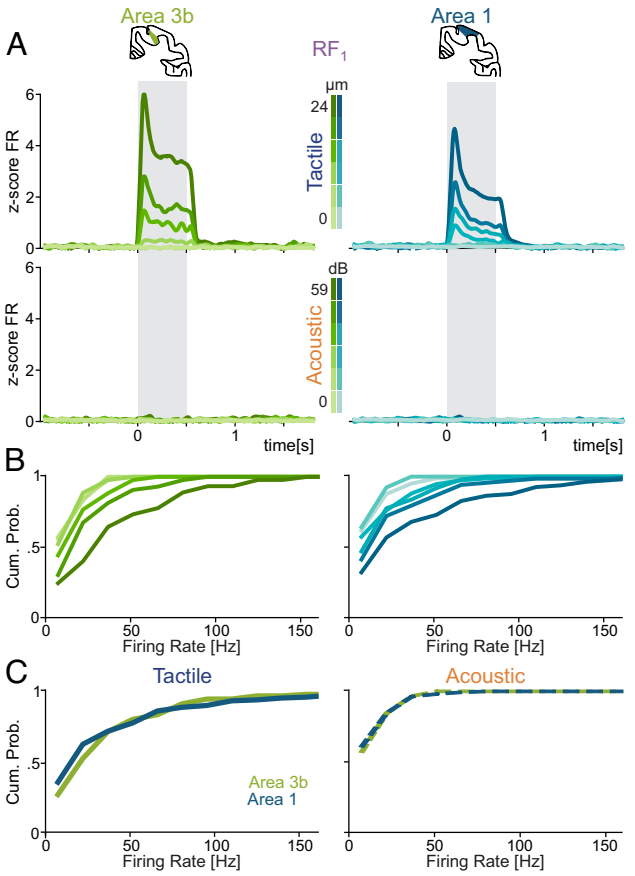

Fig. 3. (*A*) Normalized population activity for the tactile (*Top*) and acoustic (*Bottom*) conditions, obtained from neurons recorded in areas 3b (left column) and 1 (right column) in the RF_1 condition. Each colored line represents the average of normalized activity obtained from trials of the same class of stimulus. (*B*) Cumulative distributions of firing rate for the tactile modality calculated during the stimulus period for areas 3b (*Left*) and 1 (*Right*). (*C*) Cumulative distributions of the firing rate considering only the supraliminal tactile (*Left*, 12 and 24 µm) and acoustic (*Right*, 52 and 59 dB) stimuli, for neurons recorded in areas 3b and 1 ($AUROC_{tac}$ = 0.545 ± 0.037, P = 0.370; $AUROC_{acus}$ = 0.516 ± 0.029, P = 0.736).

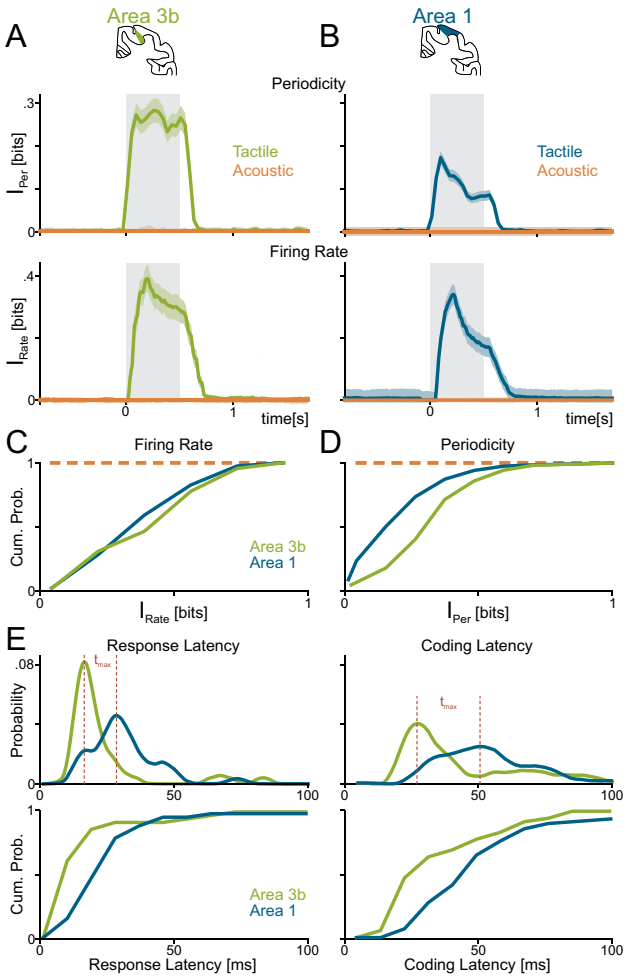

Fig. 4. (*A* and *B*) Populational mutual information between stimulus intensity and periodicity (*Top*) and firing rate (*Bottom*). Both types of mutual information were calculated by pooling neurons with RF_1 for each area and condition: A) area 3b (green for tactile) and B) area 1 (blue for tactile); the acoustic condition is shown as an orange trace in all panels. Periodicity mutual information was calculated by using the power at 20 Hz of the firing rate's spectral decomposition. (*C* and *D*) Cumulative distributions of information during the stimulation period for tactile modality (firing rate: AUROC = 0.59 ± 0.049, P = 0.1; periodicity: AUROC = 0.708 ± 0.047, P < 0.0002). (*E*) Probability distributions (*Top*) for response (*Left*) and coding (*Right*) latencies for the tactile modality in areas 3b and 1. The peaks of the probability distributions (t_{max}) were 21.14 ms in area 3b and 29.72 ms in area 1 for the response latency and 36.67 ms in area 3b and 53.25 ms in area 1 for the coding latency. Both latencies were calculated by calculating AUROCs across time bins. On the other hand, the bottom panel shows a comparison of the cumulative distributions between both areas for the response (AUROC = 0.759 ± 0.050, P < 0.0002) and coding (AUROC = 0.711 ± 0.062, P =0.002) latencies.

Rate and Periodicity as Neural Codes. To further test this hypothesis, we measured the information contained in the firing rate and periodicity of all RF_1 neurons in areas 3b and 1 using Shannon's mutual information (*SI Appendix*). We found significant differences in both rate and periodicity information when tactile stimulation was applied, while no such variations were found in the acoustic case (Fig. 4*A*). Reflecting its purely sensory nature, 3b neurons yield high values of periodicity information, while neurons from area 1 show only half the value for this metric. This loss of periodicity information suggests differences in processing between areas 3b and 1; being area 1 based on a rate code rather than periodicity code. No significant differences (AUROC: 0.585 ± 0.049, P = 0.1, n = 5,000 permutations) were found in the information that single neurons from areas 1 and 3b carried in their firing rate (Fig. 4*C*). In contrast, we found significant differences in periodicity (Fig. 4*D*) between both areas in the tactile modality (AUROC = 0.708 ± 0.047, P = 0.0002, n = 5,000 permutations). This reduction in periodicity representation between areas 3b and 1 has been previously found in a discrimination task (24), but not in a detection task.

For acoustic stimuli, no rate or periodicity information was found in either area (Fig. 4 *C* and *D*, dashed orange line). This is consistent with the results shown in the previous section where acoustic inputs do not generate any sort of activity modulation and reinforces the idea of the unimodal nature of these primary sensory areas of S1. Given the changes in periodicity information between areas 3b and 1 during tactile stimulation, we asked whether discrepancies were also present in their response latencies (RL) and coding latencies (CL). We observed that RL and CL distributions (Fig. 4*E*) were consistently left-shifted for 3b with respect to area 1. Quantification of t_{max} values for RL (21.14 ± 2.27 ms, 3b; 29.72 ±1.76 ms, 1; Upper left panel) and CL (36.67 ± 3.58 ms, 3b; 53.25 ± 3.38 ms, 1; Upper right panel) confirms this observation, exhibiting significantly smaller t_{max} values for 3b with respect to 1. We verified the significance of this shift left by computing the AUROC values across areas 3b and 1 distribution for both RL (0.759 ± 0.05, P < 0.0002; Lower left panel) and CL (0.711 ± 0.06, P = 0.0002; Lower right panel). These results strongly suggest that neurons from 3b are faster to respond and

code the identity of tactile stimuli than those in area 1. Although differences in intrinsic timescales were recently identified (17), as far as we know this is the first evidence that shows a marked difference in the processing of vibrotactile stimuli between these two sensory areas.

Variability Fluctuations during the Tactile and Acoustic Stimuli. Considering that neither firing rate nor periodicity have shown changes during acoustic stimulation, we wondered whether neural variability was modulated by acoustic stimuli. The population's distribution of Fano factor (FF) values showed no changes in either subarea during acoustic stimulation (Fig. 5 *A* and *B*). However, when tactile stimulation was presented, a clear decrease in variability (greater in area 1) was observed in both areas, highlighting once again the unimodal nature of these sensory areas. While this decrease was previously reported in visual areas (25), to our knowledge this has not been shown to occur in areas 3b and 1. Further, these results are noteworthy given that previous studies have suggested that variability modulation could be an indicator of the presence of a stimulus of another modality (26).

The variability differences between areas 3b and 1, during the stimulus period, raised the question of whether these two areas are intrinsically different at the fluctuation level. To explore such a possibility, we measured the FF and the CV during the pre-stimulus period. Noticeable differences between area distributions were detected, with significantly higher intrinsic variability in area 1 with respect to area 3b (AUROC = 0.758 ± 0.035, $P < 0.002$, n = 5,000 permutations) (Fig. 5 *C* and *D*). These results gave us the idea of the existence of a clear separation in how areas 3b and 1 process the identity of the tactile stimuli, suggesting a hierarchy in the circuit of tactile information processing, where area 3b will be then followed by area 1. Moreover, this suggests that neurons from area 1 integrate responses from more neurons than area 3b, giving rise to larger receptive fields (18) and higher variability.

To further confirm this hypothesis, we computed the firing rate autocorrelation analysis by using the neurons in both areas (Fig. 5*E*) (27, 28). Remarkably, we observed that the decay time constant (τ) was smaller for area 3b than area 1. This can be interpreted as area 1 has a greater amount of reverberations at the network level, i.e., a major level of integration (29). In summary, the results displayed up to this section provide direct evidence that areas 3b and 1 unimodally process stimuli information. Furthermore, they reveal a processing hierarchy between these two areas, with tactile stimuli information being integrated differently in area 3b than in area 1.

Are There Activity Clusters Differentiating Areas 3b from 1?. At this point, we wondered whether the differences between the responses of neurons in areas 3b and 1 were significant enough to distinguish to which area they belong to. In other words, would single neurons create two non- or low-overlapping clusters representing the two areas? Or is there an overlapping continuum of responses that connects both areas? To address this question, we performed a nonlinear dimensionality reduction on the concatenated activity profiles. The UMAP (Uniform Manifold Approximation and Projection) algorithm considers similarities at both the local and global scales (30, 31). For our data, the first UMAP dimension shows an apparent separation between the activity of both areas (*Inset* distribution Fig. 6*A*). To further test this observation, we computed a density-based metric to find clusters (32). With this procedure, we determined the existence of two density peaks (*SI Appendix*, Fig. S5*A*), one corresponding mainly to area 1 and the other to area 3b. Therefore, our analysis suggests the existence of two clusters of neurons determined purely by their responses to a set of stimuli. Based on this initial analysis, we fit a nonlinear classifier with support-vector machine (33). Fig. 6*B* shows the mean and the standard deviation of the activity profiles by using the best iteration of the classifier (0.79 ± 0.27% mean of cross-validation by using 35% of the dataset as testing). A high classification accuracy is obtained despite the clusters' unclear visual appearance, suggesting that the responses are gradually transformed from area 3b to 1. Pronounced peaks at the beginning of the stimulus period are dampened and responses to subthreshold stimuli gradually increase when transitioning from area 3b to 1 (*SI Appendix*, Fig S5*B*). This transformation may

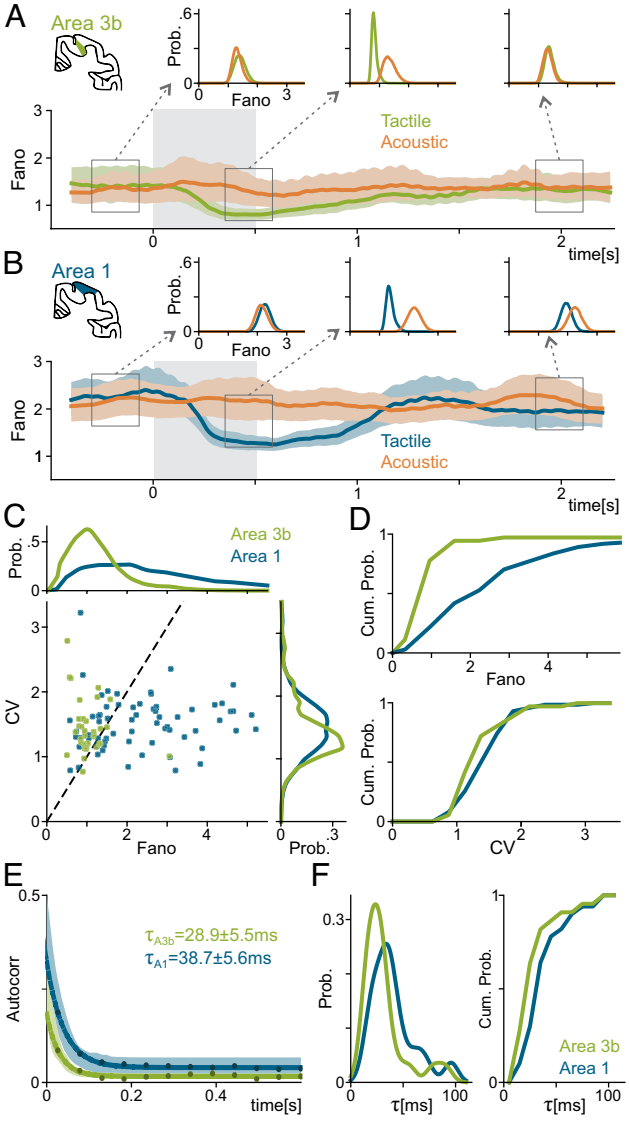

Fig. 5. Populational FF in time-dependent manner for area 3b (*A*) and 1 (*B*). FF was calculated for both modalities: tactile (green [area 3b] and blue [area 1], traces) and acoustic (orange traces). In both areas and for every modality, a set of probability distributions were calculated during a short time interval indicated by empty gray squares. Intervals displayed include the pre-stimulus period (*Left*), the end of the stimulus period (*Center*), and the delay period (*Right*). (*C*) Scatter plot comparing the coefficient of variation (CV) vs FF, during the pre-stimulus period for areas 3b and 1, accompanied with its corresponding probability distributions. (*D*) Comparison of cumulative distributions obtained for areas 3b and 1 of FF (*Top*, AUROC = 0.758 ± 0.035, $P < 0.0002$) and CV (*Bottom*, AUROC = 0.538 ± 0.038, $P = 0.478$). (*E*) Populational autocorrelation functions calculated during the pre-stimulus period yielded timescales of 28.9 ± 5.5 ms for area 3b and 38.7 ± 5.6 ms for area 1. (*F*) Probability (*Left*) and cumulative (*Right*) distributions of the timescale constants obtained in the pre-stimulus period for area 3b (green) and 1 (blue).

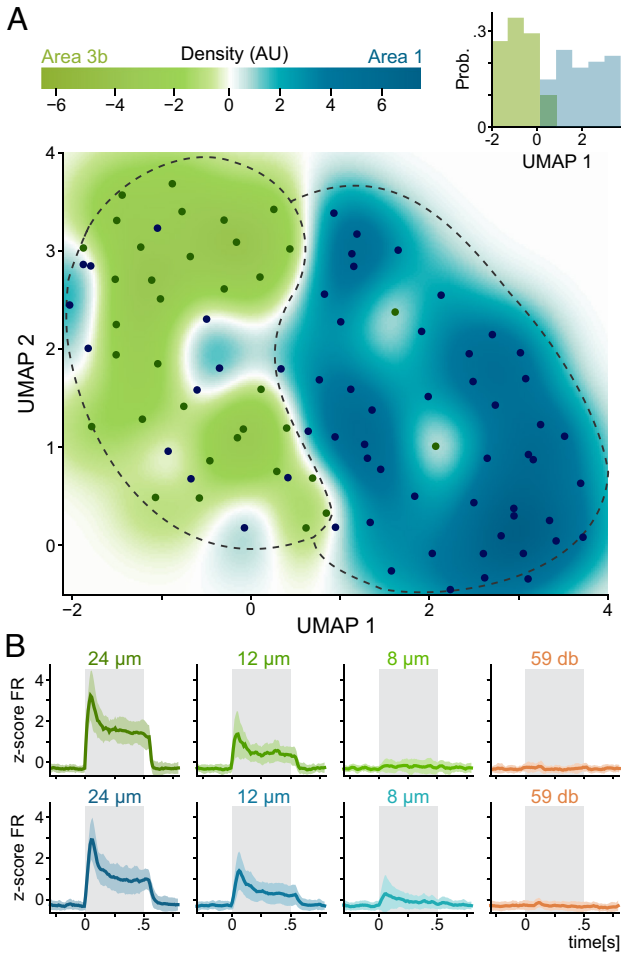

Fig. 6. (*A*) Density of 2-D nonlinear projection of the normalized neural activity obtained with UMAP (30). Green regions exhibit dominance of neurons from area 3b while blue regions show dominance for area 1. Histogram *Inset* shows the probability distribution of each area in the first UMAP dimension. (*B*) Average normalized firing rates of neurons classified with a nonlinear SVM (33). Note that most 3b neurons reside in class 1 (*Top*) and area 1 neurons in class 2 (*Bottom*). Firing rate profiles include the supraliminal (24 µm), liminal (12 µm), and subliminal (8 µm) ranges. Acoustic profiles for the supraliminal condition (59 dB), in both areas, are shown in orange. While class 1 modulated its activity with 8 µm, class 2 did not.

be related to the differences in timescale integration shown in Fig. 5. This mirrors the relationship between S1 and the ventral posterior lateral nucleus of the thalamus [VPL; ref. (34)], with area 1 neurons appearing more stable (in their coding) than those in area 3b. Even if our results are not based on and do not imply an anatomical hierarchy, our findings could lead to a more refined hierarchy of touch processing: VPL -> 3b -> 1.

Discussion

To investigate the existence of multisensory processing in S1, we recorded the neural activity of subareas 3b and 1 during a BDT where hearing and touch compete from trial to trial. By separating single units according to the relative position between their receptive field and the stimulation zone (RF_1, RF_2, and RF_3), we could study the activity response and variability of neurons whose receptive field was not directly stimulated. At the unit and population levels, we found that both areas process and encode the tactile stimulus but not the acoustic stimulus. Yet, we found three significant differences between areas 1 and 3b: 1) a decrease in periodicity information from area 3b to 1, suggesting that neurons from area 1 have a less faithful representation of the stimuli; 2) an increase in variability and timescale from area 3b to 1, indicating differences in their information processing; 3) neural responses suffer a gradual transformation between areas 3b and 1. Particularly, we observed that neurons from area 1 respond stronger to subliminal stimuli than neurons from area 3b, highlighting the differences in processing between the areas. Our results suggest that neurons from areas 3b and 1 are unimodal in their sensory processing and reveal the existence of a novel intrinsic level of processing-based hierarchy within S1.

The main characteristic of primary sensory cortices is their representation of stimulus features from the external world. Thus, to consider a response as overtly multisensory it should code the properties of stimuli from more than one modality. In contrast, our work reveals that the rate and periodicity of areas 3b and 1, at the single and populational levels, encode information unimodally. Furthermore, we found that periodicity information decreases from 3b to 1, even though these areas showed similar firing rate responses. Previous studies in a different task have shown that, while neurons from area 3b display phase-locking and periodic responses (35), the secondary somatosensory cortex (S2) also demonstrates a loss of periodicity information at the population level (24) with the construction of more complex and abstract coding (36). The role of S2 in the BDT is an important avenue for future research. Moreover, as stated in the Results section, variability analysis is a key concept that should be considered, but it has rarely been applied in the context of multisensory processing.

Even if isolated neurons demonstrate stochastic variability, they are much more reliable than those observed in brain recordings (37). Ergo, at least part of the cortical variability comes from fluctuations in synaptic inputs, shaped by the network's features (38, 39). In the mammalian brain, the degree of variability at the single-neuron level increases with the stages of sensory processing, being lowest in the periphery and highest in cortical structures (28, 40). Understanding the nature and origin of variability is imperative to the study of the neural codes used for the representation and processing of information in cortical networks (41–43). In relation to our subject of interest, a previous study has proposed that multisensory activity could be manifested in firing rate fluctuations (26). Nevertheless, we found no significant FF modulation during acoustic stimulation. Remarkably, the activity of areas 3b and 1 showed consistently significant differences between their variabilities during tactile stimulation. These differences in the neural activity: variability, response latency, coding latency, and intrinsic timescales, have all been considered indicative of differences in the hierarchy of processing (28). In somatosensory processing, a response latency hierarchy has been previously reported, beginning at thalamus and followed by S1 (44), then area 2, S2, and so on (45). Such an increase in response latency is usually accompanied by an increase in the degree of abstraction or, conversely, with a reduction of sensory information content. Both phenomena were observed in the activity of area 1 with respect to area 3b in this work. Specifically, the reduction of sensory information content was observed as a loss of periodic structure information in area 1's response. Additionally, we show that the intrinsic timescales of these areas are different in a way that is consistent with previous studies of timescale and hierarchy (17, 27, 29): the earlier area 3b has a smaller timescale than the latter area 1. In this sense, it also bears repeating that each of these areas has its own map of the whole body, and that the size of receptive fields increases from area 3b to area 1 (18, 19); this increase in receptive field size has also been related with hierarchical processing in the visual cortex (28). In sum, our findings support the notion that the

differences observed between areas 3b and 1 of S1 are explained by their different positions in the processing hierarchy.

At this point, we asked ourselves whether these two populations are different enough to reliably identify to which one a neuron belongs. To answer this, we leveraged the topological properties of the firing rates of neuronal populations using a novel manifold learning method (UMAP) (30, 31). In other reports, this algorithm has revealed interesting dynamic and geometric properties (46, 47). Our analysis revealed a clear separation of areas 3b and 1, which is nonetheless connected by a continuous transition between their neural activity profiles. This transition ignored the acoustic stimulus completely, considering only the tactile responses, particularly to low-amplitude stimuli. The discrepancy in timescales across these two subareas may be one of the network features that promote this separation. Area 1 neurons are equipped with larger receptive fields and timescales compared to neurons from area 3b, which may cause greater sensitivity to low amplitudes via sensory integration. Future simultaneous recording studies across this network should measure information flow (48) to look for more evidence to solve this question.

Historically, sensory processing in area 1 has been considered indistinguishable from that in area 3b. In our opinion, this conception is due to their similar firing rate patterns and a tendency to focus on single units rather than populations. The results shown here provide strong evidence that areas 3b and 1 are located at different levels in the somatosensory network's processing hierarchy. This is in line with recent results, where analogous distinct steps in the processing and abstraction of visual information were found (28). However, disparities in thalamocortical input between the areas provide an alternative explanation that is not accounted for in our work. Future research should focus on answering whether the processing differences found here emerge as a consequence of their network features or due to discrepancies in the amount of information projected from the thalamus.

Contemporary research in which different types of brain signals were used, such as LFP (10, 11), EEG (6, 12), or fMRI (8, 49), has tried to demonstrate that multisensory activity appears at the early stages of processing in the primary sensory cortices. Up to this point, the reader can see that the results presented in this work differ notoriously from those; we think that this difference could be due to multiple factors.

First, our analysis does not explore the fully aggregated activity of neurons as LFP or EEG do. So, if the aggregated or synaptic activity of S1 encodes some properties of acoustic stimuli might still be debated. Multisensory processing could be present in facets of the network outside the scope of our data, which could be detected using different approaches: 1) studying correlated variability at the level of spikes and/or oscillations; 2) taking LFP approaches, which are common in the study of attention phenomena and the transition between network states (50); and finally 3), if multisensory processing is present in the interaction between areas, this could be measured through phase or amplitude coherence, joint fluctuations, or interaction metrics with any combination of spikes and fields. We think that the approaches mentioned above are mandatory for a better understanding of the processing of sensory stimuli in primary sensory cortices; we will attempt to use them in future research.

Second, our task is competitive in nature; it divides attention between two senses, with their presence being mutually exclusive. This contrasts with most cognitive tasks employed in other studies, where the stimuli are synchronized and cooperative. If multisensory activity only appears in early sensory cortices during cooperative stimulation, or the even more complex naturalistic stimuli, then it might be more appropriate to conceptualize it as an effect that enhances unimodal processing. However, the exclusively competitive nature of our task does not permit us to evaluate such a hypothesis. Additionally, it is important to consider that the use of two synchronized stimuli hinders neural coding analyses due to the introduction of more variables into the coding problem. This multivariate extension is sometimes not well controlled or does not take the coding problem into account when designed, possibly yielding uninterpretable results (51).

Finally, on the subject of coding, experiments described in other works lack control at the finest level of the stimuli's physical parameters (6, 8, 10–12, 49). This makes it impossible to determine which physical properties were being encoded by neurons, which would have been indisputable proof of sensory modulation. In contrast, our experimental framework was designed to look for the most specific form of multisensory processing possible, with the physical properties of stimuli accurately controlled. In addition, performance in the BDT is scarcely impacted by dividing attention (15), making it possible to isolate the response to pure unimodal stimuli and determine unequivocally the effects of another modality in S1.

The opening question at the beginning of this paper, about a car's radio, is most often used as an example of attention. As mentioned briefly there, attention gives an alternative hypothesis to multisensory processing; attentional mechanisms could contribute to the differences between neural responses in unimodal and those in multimodal tasks. We also mentioned that our task should not be affected by these mechanisms (15). Still, it is relevant to discuss some of the findings that relate attention to the topics of our study. For example, attentional effects have been evaluated in circuits near the primary sensory areas, but it remains unclear how attentional phenomena affect primary processing (52, 53). It has also been reported that multisensory processing could be task-dependent (54, 55). This could point to more elaborate interactions between the hypothetical early multisensory integration, attention, and task demands. The study of these interactions could greatly benefit from the synthetic approach advocated in (56); there, the concept of attention was critiqued for being too broad for the study of neural systems. Rather, the approach advocated involves proposing specific stimuli selection processes relevant to behavior. In any case, the experiment presented here is not optimal for the study of attentional effects. For this purpose, a better experimental design based on the BDT could consist of adding two more stimuli: one serving as a cue, prior to the original stimulus, indicating to the subjects which modality to attend; and the other, synchronized with the original, acting as a distractor. With this task, it would be possible to disentangle the effects of attention, uncertainty, and multisensory processing in the activity of the primary sensory cortices, while also increasing the competition between modalities.

In brief, we did not find evidence in favor of acoustic processing at the level of firing rate nor variability for areas 3b and 1 of S1, no matter the relative location of the neuron's receptive field stimulated during the BDT. Remarkably, we found clear differences between areas 3b and 1 at the level of variability, periodicity information, and timescales during the tactile stimulus. In addition, utilizing a powerful nonlinear dimensionality reduction technique we found a clear, but continuously connected, separation between these two areas.

Materials and Methods

Two monkeys were trained to detect the presence or absence of a single vibrotactile or acoustic stimulus (Fig. 1*A* and *SI Appendix*). Neuronal recordings were obtained in the primary somatosensory cortex (S1) while the monkeys performed the BDT. Animals were handled in accordance with standards of the NIH and

Society for Neuroscience. All protocols were approved by the Institutional Animal Care and Use Committee of the Instituto de Fisiología Celular, Universidad Nacional Autónoma de México.

Data, Materials, and Software Availability. Data files are publicly available at Zenodo (https://zenodo.org/record/7293495); see ref. (57).

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