



Supporting Online Material for
Temporal and Spatial Enumeration Processes in the Primate Parietal Cortex

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Supplementary Online Material

Temporal and Spatial Enumeration Processes in the Primate Parietal Cortex

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Methods

Stimuli. The items were black dots (diameter range 0.5 to 1.1 deg of visual angle) displayed on a gray background (diameter: 6 deg of visual angle). Successive dots (separated by short pauses during which only the gray background was visible) in the sequential protocol were presented in the center of the display, whereas the multiple dots in the simultaneous protocol were randomly arranged (see **Fig. 1a,b**). To prevent the monkeys from memorizing the visual patterns of the displays, each quantity was tested with 100 different images per session and the sample and test displays that appeared on every trial were never identical. All four quantities of items were used in each session and all displays were newly generated for each session by pseudo-randomly shuffling relevant item features (e.g., position and size in the multiple-item displays). Both the simultaneous and the sequential protocol were applied in each session with one standard and one control condition per protocol and appeared in random order with equal probability ($p = 0.25$). Non-numerical spatial and temporal cues were controlled across different quantities (**Table 1** and **Fig S1**).

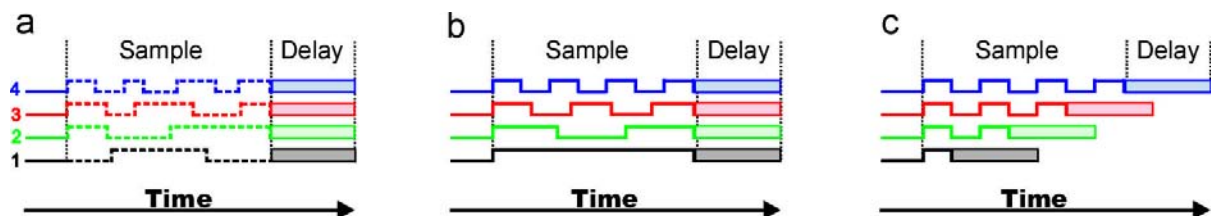


Fig. S1: Stimulus types for the sequential protocol followed by a constant delay period. **(a)** In the 'standard protocol', the duration of individual items and pauses in the sample period was pseudo-randomized from trial to trial (dotted lines in the stimulation illustration represent an example presentation layout) within a sample period of constant duration across numerosities. **(b)** In the 'equal sample duration' protocol, individual items and pauses had a constant duration for any given numerosity to match the constant duration of the entire sample period. **(c)** The duration of the items and pauses was equal across numerosities in the 'equal item/pause duration' protocol. Here, the duration of the sample period grew in proportion with the number of items. Monkeys had to match sequentially presented numerosities to numerosity in multiple-item displays.

Behavioral protocol. A trial started when the monkey grasped a lever and fixated a central fixation target. After a 500 ms pure fixation period, the sample display period started, which lasted 800 ms in the simultaneous protocol. The sample period in the sequential protocol lasted 2500 ms; in few sessions the sample duration was extended to 3500 ms to further increase temporal variation. A constant 1000 ms memory delay followed. Next, a test

display appeared (always a multiple-dot display), which in 50 % of the cases was a match showing the same number of items as the sample period (match-trials). In the other 50 % of the cases (non-match trials) the first test display after the delay period was a non-match (it contained – with equal probability - either more or less items in the multiple-dot display, except for trials with sample numerosity “one”) followed by a second test display which always was a match. If a match appeared, monkeys released the lever to receive a fluid reward. If a non-match was shown, they held the lever until the second test display appeared (which in these trials was always a match) requiring a lever release for a reward. Thus, the monkeys made the actual decision whether to release or maintain the lever during the presentation of the first test display (see **Fig. 1a,b**). Trials were randomized and balanced across all relevant features (e.g., match vs. non-match, sequential versus simultaneous, etc). **Fig. S2** shows the detailed behavioral performance functions of both monkeys in the sequential and the simultaneous protocol. Monkeys had to keep their gaze within 1.75 degree of the fixation point during sample presentation and the memory delay (monitored with an infrared eye tracking system).

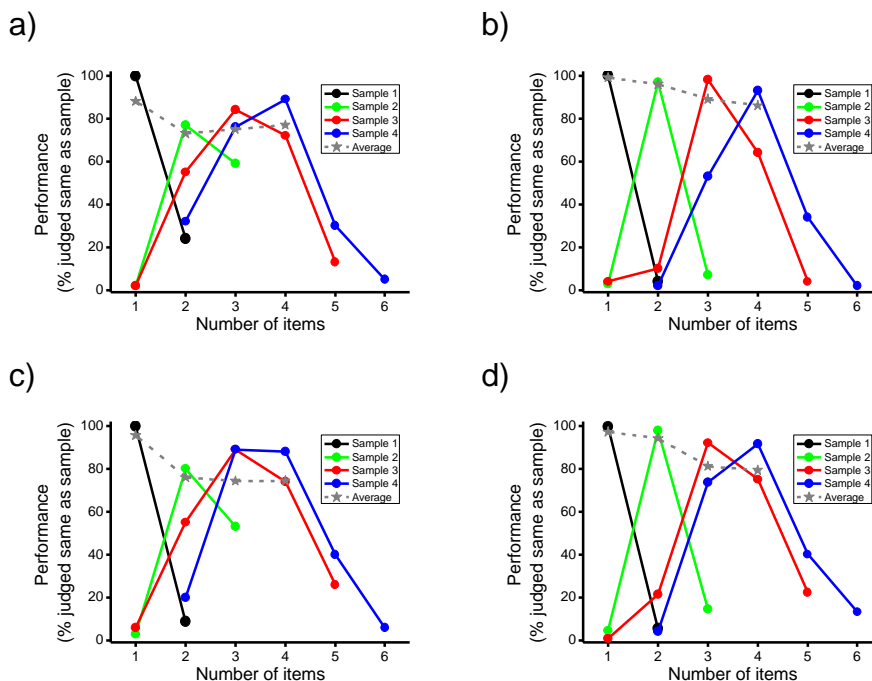


Fig. S2: Behavioral performance curves of monkey W (**a,b**) and monkey R (**c,d**) to the sequential (**a,c**) and simultaneous (**b,d**) stimulus protocols. The functions indicate the probability that a monkey judged displays in the test period as containing the same number of items as the sample numerosity. The center data point of each colored function indicates the correct performance in the match trials (where the first test display showed the same numerosity as had been cued in the sample period) for the four sample numerosities (see figure legend). The data points to the left and the right of the center indicate performance in the non-match trials (i.e., where the first test display showed a smaller or larger number of items); for the non-match numerosities the percentage of errors for the respective non-match numerosity is plotted. The red curve in **a**), for example, represents all trials with ‘three’ as sample numerosity. The monkey judged correctly in 84 % of the presentations numerosity ‘three’ (center of the function) in the first test display as matching the sample numerosity (namely ‘three’). When the non-match numerosity ‘two’ and ‘one’ appeared in the first test display, the monkey released the lever to indicate a numerical match in 55 % and 2 % of the trials, respectively, thus causing errors. Similarly, the monkey released the lever to the non-match numerosity ‘four’ and ‘five’ in the first test display to indicate a numerical match in 72 % and 13 % of the trials, respectively, thus causing again errors. The functions illustrate the numerical distance effect, i.e., it is more difficult for the monkey to discriminate close numerosities (3 versus 2 and 3 versus 4, in this example) than numerosities that are remote from each other (3 versus 1 and 3 versus 5).

Training procedure. Both monkeys were first trained on the simultaneous protocol until they discriminated multiple-dot patterns reliably according to the number of items. Subsequently, they were gradually trained to enumerate sequential items throughout the course of several weeks. Monkeys could not be expected to immediately understand the logic of the sequential protocol because it had a completely different temporal design. We thus did not incorporate any transfer tests from simultaneous to sequential numerosity presentation.

Initially, the monkeys learned to discriminate only sequential numerosity 2 versus 4 (i.e., 4 was the non-match for sample 2, and 2 was the non-match for sample 4). To test whether they would grasp the concept of sequential numerosity after this basic training with 2 versus 4 sequential items, we tested the monkeys in transfer tests. Throughout the ongoing reinforced 2 versus 4 discrimination, we occasionally ($p = 0.1$) inserted transfer trials showing three consecutive items in the sample period. Non-match numerosities for transfer numerosity 3 were 2 and 4. Even though they were not reinforced for any particular response to sequential numerosity 3 (i.e., rewarded at chance), they discriminated sequential numerosity 3 from 2 and 4 with an accuracy comparable to that for the baseline discrimination for 2 and 4 (see **Fig. 1c,d**). After that, the entire range of sequential numerosities from 1 to 4 was introduced and correct responses were reinforced. Finally, the simultaneous and sequential protocols were mixed within a session. Recordings started after the animals performed both the sequential and simultaneous protocols and fixated reliably.

Recording method. Recordings were made from one left and one right hemisphere in the depth of the intraparietal sulcus (IPS) of two rhesus monkeys (*Macaca mulatta*) in accordance with the guidelines for animal experimentation approved by the Regierungspräsidium Tübingen, Germany. This area was chosen because it contains the highest proportion of visual numerosity selective neurons (23) and is specifically activated by quantity information in humans (17,19,21). Arrays of four to eight tungsten microelectrodes (1–2 M Ω impedance) were inserted using a grid with 1-mm spacing. Recordings from the IPS were exclusively done at depths ranging from 9 mm to 13 mm below the cortical surface. Electrodes were advanced roughly perpendicular to the cortical surface passing through the lateral or medial bank. Recordings were localized using stereotaxic reconstructions from magnetic resonance images. The Horsley-Clark coordinates of the IPS recordings ranged from 2 mm posterior to 3 mm anterior (see “Visual direction selectivity of neurons”). Both monkeys are still engaged in quantity discrimination studies. Neurons were selected at random; no attempt was made to search for any task-related activity. Separation of single-unit waveforms was performed off-line applying mainly principal component analysis.

Data analysis. In the simultaneous condition, sample activity was derived from an 800 ms interval after stimulus onset shifted by a cell’s individual response latency. In the sequential condition, the spike rate to each individual item in the sample period was measured after deriving the precise onset and duration of any given item on a trial-by-trial basis (for the standard sequential protocol, single items’ onsets and durations were pseudo-randomly chosen by the computer program prior to each single presentation). Again, the analysis window for every item was shifted by the neurons’ response latency. To measure neuronal response latency, we generated average spike density histograms (at 1 ms resolution, smoothed by a sliding window, kernel bin width: 10 ms) for a neuron’s responses to all sample stimuli. Discharges following sample onset were compared to spike rates in a 200 ms interval preceding sample onset. Response latency was defined by the first time bin that reached a value higher or lower than any value before sample onset. A default latency of 100 ms was

used if no measure based on these criteria could be derived. For the delay period, activity was summed in a 800 ms interval starting 200 ms after delay onset.

To determine numerosity-selectivity for the sample period in the sequential and simultaneous protocol separately, a two-factorial analysis of variance (ANOVA) was calculated with numerosity (one to four) and stimulation condition (standard or control) as factors. In the delay period, a two-factorial ANOVA was computed with numerosity (one to four) and stimulus protocol (sequential or simultaneous) as factors. All ANOVAs were calculated with the square-root transformed spike rates values to render spike rate distributions normal and to equalize the population variances (*I*). ANOVAs were calculated separately for sequences of two, three and four items. A neuron was judged to be numerosity selective in the sequential protocol if the preferred numerosity was the same in all separate analyses for two, three and four items in a sequence. Numerosity-selectivity in the simultaneous protocol was derived during the 800 ms sample period (**Fig. S3**).

To derive averaged numerosity-filter functions, the tuning functions of individual neurons were normalized by setting the maximum activity to the most preferred quantity as 100 % and the activity to the least preferred quantity as 0 %. Pooling the resulting normalized tuning curves resulted in averaged numerosity-filter functions.

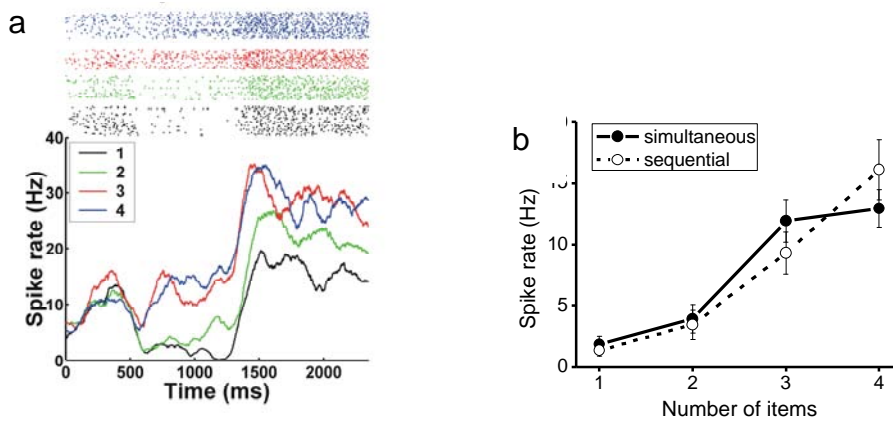


Fig. S3: Responses of the neuron shown in Fig. 2f-h to the simultaneous protocol. (a) Dot-raster histogram and spike-density histogram derived from the discharges to multiple-item displays. (Sample onset at 500 ms, delay onset at 1300 ms). This neuron belonged to one of the two cells tuned to the same numerosity ‘four’ in both the simultaneous and the sequential protocol as indicated by the tuning functions (b). This neuron was also selective during the delay period.

Multiple regression analysis. To statistically assess whether temporal parameters influenced the activity of numerosity-selective neurons to their preferred sequential item, we performed a multiple linear regression analysis using the following regression model:

$$y = \beta_0 + \beta_1 * x_1 + \beta_2 * x_2 + \beta_3 * x_3$$

where y is the discharge rate to the preferred sequential item, x_1 is the duration of the preferred item in a sequence, x_2 is the duration of the pause preceding the preferred item, and x_3 is the duration of the previous-to-preferred item. (For neurons selective to the first item, a simple linear regression was applied). Furthermore, β_0 is the intercept, and β_{1-3} are the corresponding regression coefficients.

We calculated the probability that at least one of the coefficients equalled zero by an F-statistic. Furthermore, we got the significance values for each parameter by a t-test. If at least

one of the tests was significant with $p < 0.01$, the neuronal activity was accepted as reflecting non-numerical temporal factors and the cell was excluded from the pool of numerosity-selective neurons. Correlation coefficients for discharges to the preferred sequential item as a function of the above temporal parameters are displayed in **Fig. S4**.

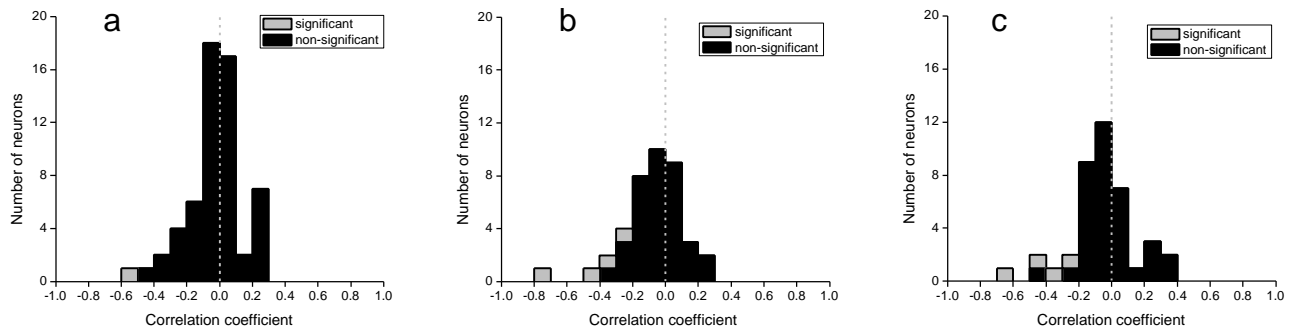


Fig. S4: Correlation coefficients derived from multiple linear regression analysis to test the influence of temporal factors on the discharge of neurons selective for sequential numerosity. Frequency histograms of correlations coefficients for (a) the duration of the preferred item in a sequence, (b) the duration of the pause preceding the preferred item, and (c) the duration of the previous-to-preferred item are plotted. Few of the neurons discharged significantly as a function of temporal parameters.

With the same regression model, we tested the influence of temporal parameters on the discharges to the preferred pause in between sequential items. In this case, however, y is the discharge rate to the preferred pause in between sequential items, x_1 is the duration of the preferred pause in a sequence, x_2 is the duration of the item preceding the preferred pause, and x_3 is the duration of the previous-to-preferred pause. Correlation coefficients for discharges to the preferred sequential pause as a function of the above temporal parameters are displayed in **Fig. S5**.

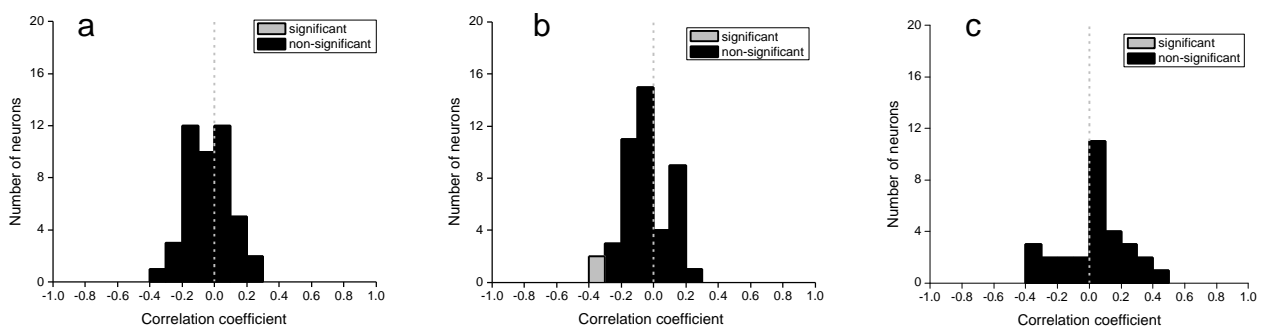


Fig. S5: Correlation coefficients derived from multiple linear regression analysis to test the influence of temporal factors on the discharge of neurons selective for pauses between sequentially displayed items. Frequency histograms of correlations coefficients for (a) the duration of the preferred pause in a sequence, (b) the duration of the item preceding the preferred pause, and (c) the duration of the previous-to-preferred pause are plotted. Only two of the neurons discharged significantly as a function of the duration of the item preceding the preferred pause.

Test for visual motion direction selectivity. Many neurons were additionally tested with flow field stimuli to investigate the location of numerosity-selective recordings sites relative to ventral intraparietal area (VIP), an area containing a high proportion of visual direction selective cells (2). The stimuli consisted of white randomly distributed dots (0.06 deg. of vis. angle, 10 % density) coherently moving on a circular black background (23 deg. of vis. angle) in one of 6 possible directions (up, down, left, right at a speed of 5.4 deg. of vis. angle; approaching and receding at a speed of 9 deg. of vis. angle). The flow field stimuli were presented for 500 ms at a 60 Hz refresh rate. The animals were rewarded for passively viewing the stimuli while maintaining gaze fixation within 1.5 degree of the fixation point. Blocks of flow field stimulus presentation were randomly interleaved throughout the ongoing numerosity discrimination. Neurons were included in the analysis if at least six repetitions of each motion direction could be presented. Neuronal responses were derived in 500 ms windows (shifted by the neurons' individual latencies relative to motion onset, or by a default 80 ms for those neurons where latency could not be determined) and statistically evaluated by a Kruskal-Wallis test at criterion of $p < 0.05$.

Sufficient stimulus repetitions could be presented at 114 recording sites in the fundus of the IPS (see "Recording method" for anatomical coordinates). At 63 of these sites, one or more single-units were found (based on spike sorting) that were significantly tuned to motion direction (**Fig. S6**); thus 55 % of our recording sites exhibited visual motion direction selectivity, arguing for VIP-recordings. Note that our proportion of motion direction selective neurons constitutes a conservative estimate based on the relatively few numbers of repetitions and the restricted range of motion directions and speeds (which was due to the time constraints placed by the actual numerosity task). Note also that our recordings were completely unbiased with respect to the neurons' response properties; every neuron that could be well isolated was incorporated into this analysis. Interestingly, we often recorded pairs or triplets of neurons at the same electrode that responded differently to the motion patterns. In 33 recording sites where at least two units could be isolated, 9 of them (27%) had all their units selective for motion direction (but rarely to the same direction), the other 24 (72 %) had at least one unit which was not tuned to any direction (**Fig. S7**).

In addition to this quantitative evaluation of motion direction selectivity, we often tested qualitatively for responses to tactile stimulation by touching different parts of the monkeys' heads; we frequently detected correlated discharges when touching head parts (primarily contralateral to the recording site). Since VIP neurons are also characterized by somatosensory responses (3), this is another indication that we recorded in area VIP.

We managed to test 75 cells that turned out to be tuned to numerosity (in any trial period) for visual direction selectivity. Roughly half of them (38/75 or 51 %) were both motion direction selective and numerosity selective. This argues for partly overlapping neuronal networks engaged in motion direction and numerical information processing.

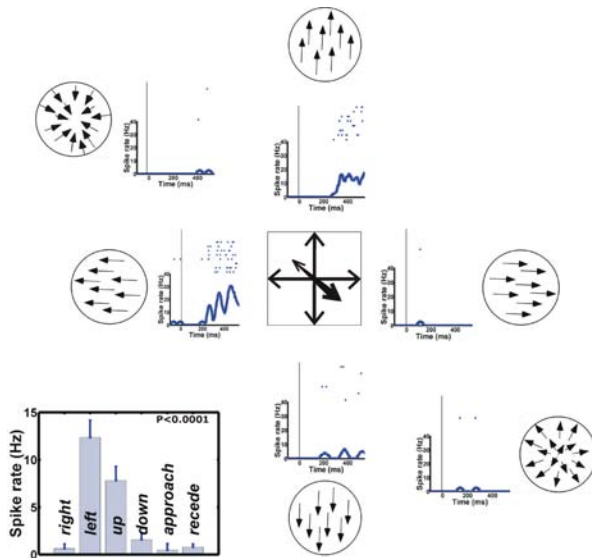


Fig. S6: Neuron in the fundus of the IPS showing a high degree of visual direction selectivity to flow field stimuli with leftward motion. The center panel illustrates the motion direction of the dot patterns. The neuronal responses for eight repetitions of each stimulus (see arrow drawings) are shown as dot-raster histograms (top panels) and averaged spike density histograms (bottom panels). Time 0 ms (vertical line) indicates motion onset; each stimulus was shown for 500 ms. The column plot in the left lower corner shows the mean discharge (error bars represent SEM) to the six directions; the p-value of the Kruskal-Wallis test indicates highly significant selectivity. This neuron was not numerosity-selective.

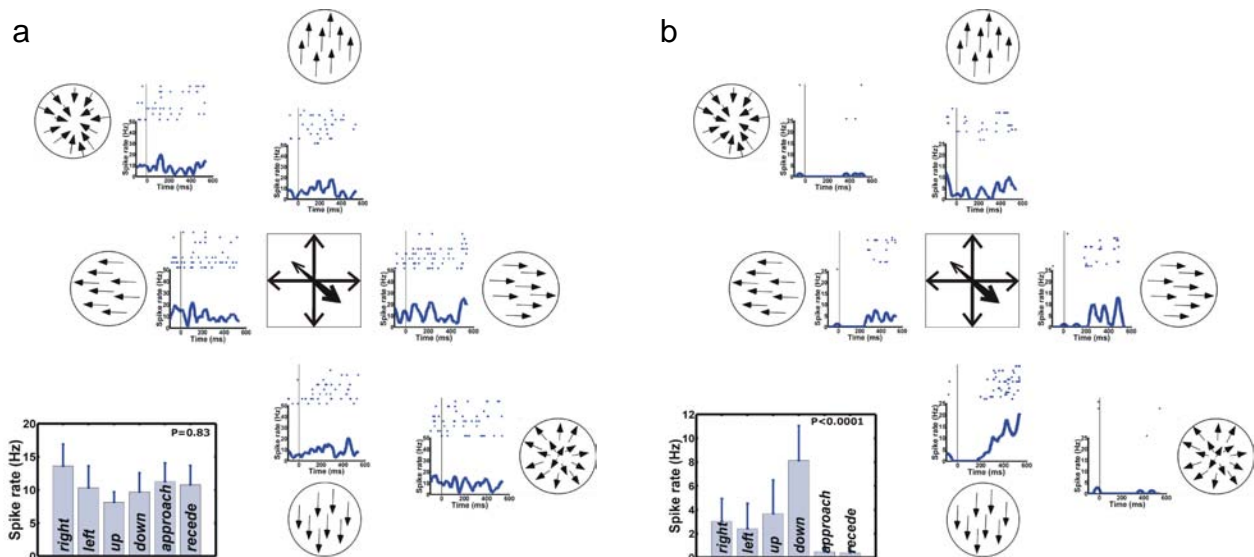


Fig. S7: Two neurons recorded simultaneously at the same electrode (layout as in Fig. S6). **a)** The single unit is the same as in Fig. 2f, which was selectively tuned to sample numerosity ‘four’ in the sequential protocol. This neuron was not tuned to visual motion direction. **b)** Interestingly, an immediately adjacent neuron recorded at the same location exhibited strong directionality (to downward motion), but no tuning to numerosity.

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