

## Reading the Neural Code: What do Spikes Mean for Behavior?

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**The present study reveals the existence of an intrinsic spatial code within neuronal spikes that predicts behavior. As rats learnt a T-maze procedural task, simultaneous changes in temporal occurrence of spikes and spike directivity are evidenced in “expert” neurons. While the number of spikes between the tone delivery and the beginning of turn phase reduced with learning, the generated spikes between these two events acquired behavioral meaning that is of highest value for action selection. Spike directivity is thus a hidden feature that reveals the semantics of each spike and in the current experiment, predicts the correct turn that the animal would subsequently make to obtain reward. Semantic representation of behavior can then be revealed as modulations in spike directivity during the time. This predictability of observed behavior based on subtle changes in spike directivity represents an important step towards reading and understanding the underlying neural code.**

The idea that neurons communicate electrically<sup>1,2</sup> the generation of spikes and the relationship of spikes to behavior has been the central theme of research for many years. Typically, spikes are perceived to have stereotyped waveforms that can be reduced to an all or none event<sup>3-9</sup>. Such reduction allowed linking of neuronal activity to behavior on a temporal basis<sup>10-12</sup>. Thus, temporal analysis of spike trains became a standard method to understand the relationship of spiking to behavior. Indeed, searching for meaning in neural activity started even earlier<sup>13</sup>. Spatial location of the rat was associated with changes in firing rates in so called "place cells" from hippocampus. However, recent investigations show that place cells in the hippocampus appear to alter their preferred firing even in the absence of any changes in the environment<sup>14</sup>. The predictability of the animal's location based on firing in the “place cells” may therefore not be as robust as once thought and such temporal analyses may be too restrictive in their ability to define the complexity of neuronal activity.

Beyond their time of occurrence, each spike can be characterized by a new feature called spike directivity. The spike directivity term can be described by a preferred direction of propagation of electrical signal during each action potential (AP) and approximated with a vector<sup>15</sup>. This characteristic can be obtained using tetrodes or any other type of multi-tip receiver (with number of tips  $n \geq 4$ ) but cannot be measured based on single electrodes techniques. The richness and importance of spike directivity to organize with learning was shown in striatal medium spiny neurons<sup>16</sup>. Such analyses add an important level of subtlety to information processing by these neurons<sup>17</sup>.

Another accepted phenomenon is that of charge movement occurring during the action potential (AP) itself. Indeed, Oesch et al.<sup>18</sup>, using patch-clamp recordings and two-photon calcium imaging demonstrated that dendrites are selective to AP directivity. Equally, the dendritic architecture has an influence on the cell response and these slight spatial changes in spike directivity in several spikes may be determined by a preferential activation of dendritic tree during each AP<sup>19</sup>.

The role of the striatum in learning stimulus-response associations, action selection or habits formation is unquestionable and has been discussed and demonstrated in several papers<sup>22,11,23,24,25,12</sup>. The dorsal striatum which receives a substantial component of its input from the sensorimotor cortex plays a central role in behavioral action selection studies<sup>11,26,12</sup>. It is for this reason that the dorso-lateral striatum was chosen in the current study to examine the relationship between observed behavior and spike directivity properties from neurons.

The presence of the so called “expert” neurons in the striatum has been advanced for some time<sup>11,20,21</sup>. The term “expert” neuron was used before in Barnes et al., relating spike timing of neurons from the striatum to meaningful events of the task on the T-maze<sup>12</sup>. We have already shown that using a charge movement model (CMM) it is possible to compute spike directivity for every action potential<sup>15</sup>. Additionally, performing information theoretic analyses, we have been able to reveal that during rewarded T-maze learning tasks, spike directivities of “expert” neurons from the dorso-lateral striatum become organized with behavioral learning<sup>16</sup>. The important question is what the newly described feature of spike directivity adds in further related to behavior.











The current paper describes the value of this organization of spike directivity in terms of what it represents within behavior. In these recordings, every tetrode implanted in the dorsolateral striatum is considered to form a frame of reference over which the electrical flow of charges in space can be analysed during each spike and trains of spikes. In this view, the directivity property of a spike is embedded in a vector that reflects the flow of

charges over the frame of reference (tetrode) for every spike. Having already shown the organization of directivity as rats learnt a procedural task<sup>16</sup> we embarked on finding the semantic relevance of this organizational process by analyzing the T-maze data during the time that is of highest value for action selection in order to obtain the reward. This is the time between the cue (tone) delivery and the beginning of turn where spiking activity of striatal neurons is reduced with learning<sup>11,12</sup>. We show that in animals which have already acquired the task, after tone delivery, spatial modulation of spike directivity (during this time of highest value) predicts the correct turn that the animal would make in order to get the reward.

## Results

During T-maze sessions (Fig 1, a) all three animals showed the expected improvement revealed in average percentages of correct responses during behavioral learning (Fig 1, b, Supplementary Methods). We recorded a daily average of 30 units per animal with six tetrodes each. Events associated with hexadecimal values are recorded simultaneously with neuronal spikes during each trial and color coded for display purposes (Table1).

The neurons showed an increase in spiking activity visibly correlated with behavioral events (tone cue, turning on T-maze, etc., Figure 2a and b). The percentage of units that responded to events in the task increased from approximately 50% to a maximum of 85% of recorded units, corresponding to a  $\chi^2$  value with  $P < 0.001$ . The number of units that responded to more than one event rose from 40% to 60%. At the beginning of training, 27% of task-related units responded during turns while by the end of training only 14% responded ( $P < 0.001$ ).

Table 1: Relationship between events and recorded values during left and right trials		
	Left	Right
Start		
Tone		
Turn Begins		
Turn Ends		
Goal		

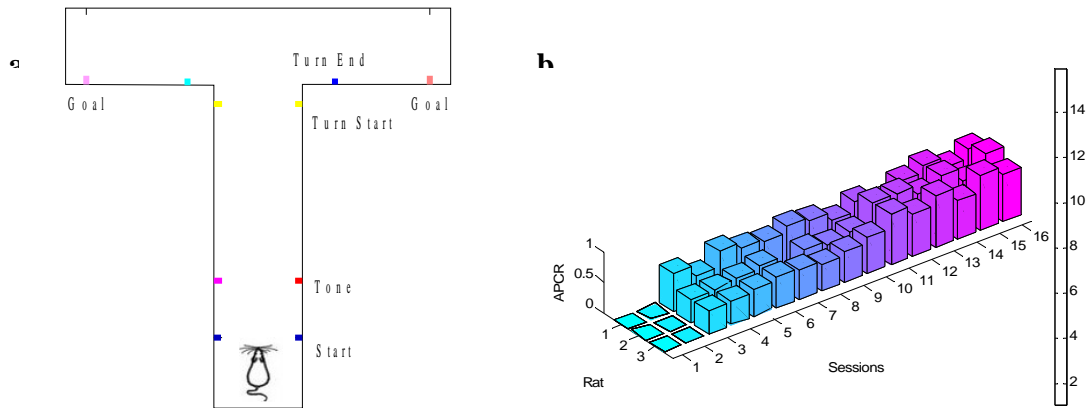


Figure 1: Experimental T-maze and behavioral response.

**a**, Schematic representation of the T maze experiment. Events are associated with colors as per Table 1.

**b**, An example of behavioral response that shows average percentages of correct responses (APCR). The cyan color shows low levels of performance while the magenta color shows at least 70% correct responses in the three rats. The vertical bar shows the color scheme used to show the change in performance level across the. Between the events of tone delivery and turn beginning, with learning, the number of spiking units decreased as did the actual number of spikes per unit occurring in this time period confirming previously published results<sup>11,12</sup>. However, the remaining responsive neurons showed increased modulation in spatial directivity with learning as shown below.

All data analysed and shown below are after at least 70% correct response stage is achieved. An example of temporal representation of events as recorded from one of the many neurons is shown (Fig 2, a). The lower half of the frame shows event flags as a color coded bar (per Table 1). The graph above represents actual spikes from this neuron in a single trial, plotted in each behavioral period. Fig 2a shows neuronal activity from the same neuron for a left turn while Fig 2b shows the right turn. The filtered version of unit activation  $s_B$  for the selected neuron is plotted in red color (Supplementary Methods). Neuronal activity occurs upon signal tone delivery, during turning movements on the right or on the left of the T-maze and before or during attaining the goal. It is also clear that the same neuron shows activity for both turns. At the end of the learning period, all neurons that are linked to turn showed this dual turn related responsivity. Other neurons showed a diversity of activities including response to start, tone or goal (analysed but not shown). Such analysis reveals that a single neuron can respond to multiple complex events within a task.

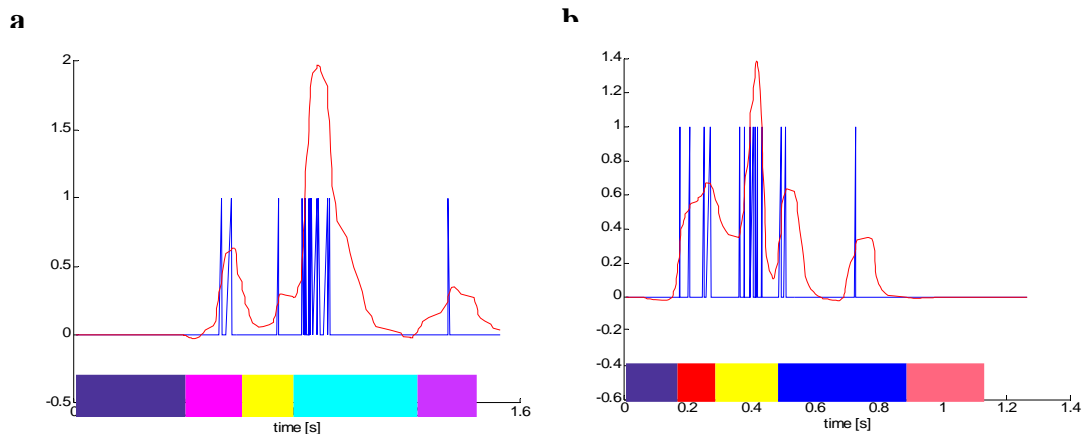


Figure 2: The variation in spike occurrence, estimated spike activity over time and events. Both graphs present activity from the same expert neuron. Event flags are represented as a color coded bar (per Table 1).

**a**, An example of temporal representation of events (bottom) where low tone was associated with left arm reward. Occurrence of spikes for the expert neuron is shown in blue color and the estimation of spiking activity in red color.

**b**, An example of temporal representation of events (bottom) where high tone was associated with right arm reward. Occurrence of spikes for the expert neuron is shown

Spatial directivity is now calculated for neurons spiking during different phases of the T-maze task. Every spike yields only one vector<sup>15</sup>. It is important to note that spike directivity analysis is carried out on neurons after the animal has already acquired the task and organization in spike directivity has occurred. The fact that directivity is reorganized during learning has already been demonstrated<sup>16</sup>.

The change of direction cosines during the whole trial is first computed in selected neurons for left and right turns. An example of these shapes for cosines angle variations is presented in Figs 3 a, b for the left and for the right turn trials on T-maze. At least two distinctive parts can be easily seen to exist in these plots for both the right and left turn trials. This characteristic profile is akin to a signature. In each trace of the change in cosines angle during the task, the first change occurs in  $\Delta\theta^L$  or  $\Delta\theta^R$  angle during or

after tone cue event (see Supplementary Methods). The second distinctive change in  $\Delta\theta^L$  or  $\Delta\theta^R$  angle appears before or during left or right turns. The change of angle during movement shows similar variability during several trials.

Supplementary Figs 1 a, b show the cosines angle changes from the same neuron in three successive trials for both right and left turns while Supplementary Figs 1 c and d show corresponding spikes (for which the directivity analysis was performed) in blue color and spike activity estimates in red color. An important observation here is that the profiles for left versus right turns are visibly similar with slight changes from trial to trial, both in the temporal and the spatial directivity plots.

Finally, these changes in the cosines angle can be compared to what happens in the traditional, spike timing view during a trial. This comparison allows us to reveal powerful similarities in dynamic variations in spike directivity and temporal activity. A correspondence between changes in the firing rate and modulation of spike directivity appears to exist as seen in Supplementary Figs 1 a-d. Thus when a significant change is seen in the timing of spike activity during a trial, there is a corresponding change in cosines angle plotted for spikes (Supplementary Figs 1: a versus c; b versus d). Three such trials are represented for the left turn and right turn trials respectively. The variation in cosines angle for every trial is similar to the corresponding changes in timing of spike occurrence.

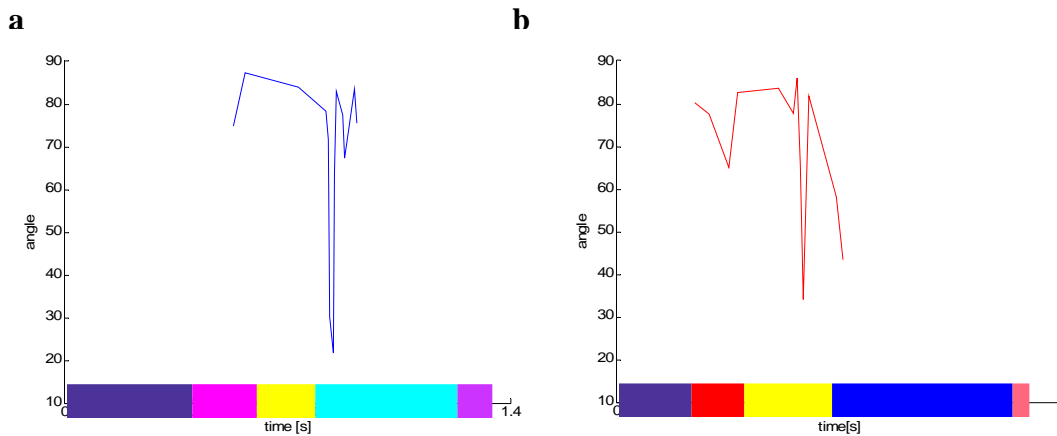


Figure 3: Changes in cosines angle in an expert neuron from striatum as related to events (bottom) over time during the whole trial. Event flags are represented as a bar, color coded as in Table 1.

**a**, An example of temporal representation of events (bottom) with low tone for turning left and variation of directivity angle in blue color.

**b**, Temporal representation of events (bottom) with high tone for turning right and variation of directivity angle in red color.

The directivity vector of every spike occurring between start and tone (Figure 4, a), tone and beginning of turn (Figure 4, b) is plotted for about 20 trials and between beginning of turn and goal (Figure 4, c) for a single trial. Sequential spikes from the same neuron during a specified turn show variability in directivity as shown in Fig 3. A principal component analysis (PCA) is then performed on a selected cosines angle yielding two distinct clusters<sup>16</sup>. These clusters are represented in specific colors for each type of turn (yellow and blue for right, red and magenta for left). Then, during a session, yellow and blue vectors can be plotted to represent these directivities for the right turn related trials on the maze. In a similar way red and magenta arrows are drawn for left turn related trials on the maze. Rotation of the three obtained 3D images showed a separation for spike directivity associated with the left versus the right turn trials on the T-maze only in Fig 4b. This separation between left and right turn related spikes cannot be seen in Figs 4a or 4c in any of the analysed neurons. It is important to note that for Fig 4b directivity is calculated for spikes between tone delivery and before the turn has actually begun and not for those spikes that occur during the actual turn.

Once learning is achieved, neurons from striatum significantly reduced their number of spikes between when the gate is open and turn is performed while the number of spikes between the turn and goal phase show an increase. This temporal reorganization

phenomenon of neuronal activity was previously described in detail<sup>11,12</sup> and can be directly observed in the number of spikes represented in Fig 4c and Figs 3a, b. While spike directivity for the data between when the gate opens and turn can be computed for

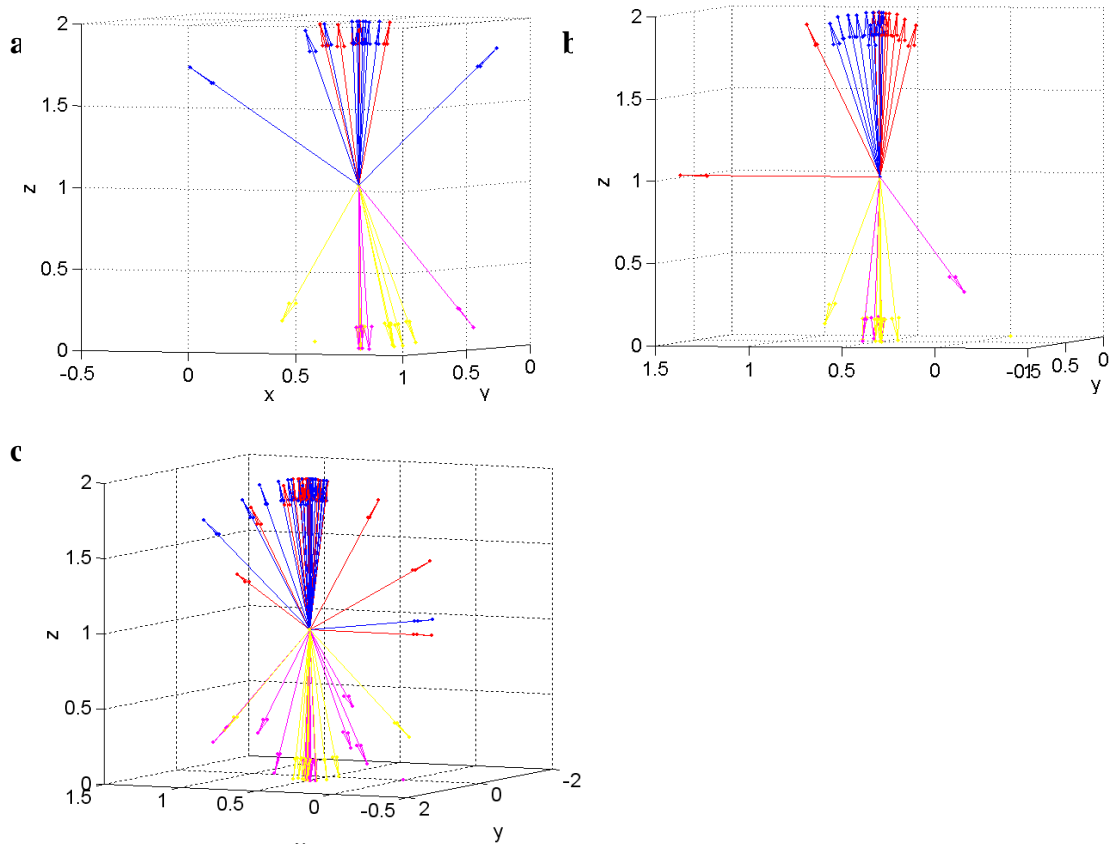


Figure 4: Representation of spike directivity vectors for spikes in an expert neuron. Left turn trial is represented by yellow and blue arrows while the right turn trial is represented by red and magenta arrows.

**a**, Spike directivity vectors of all spikes between gate opens and tone in about 20 trials

**b**, Spike directivity vectors of all spikes between tone and turn starts in about 20 trials

**c**. Representative spike directivity vectors of all spikes between beginning of turn and

every spike and represented as vectors (Fig 3 a, b), a reliable statistical analysis of these data is considerably limited due to the low numbers of spikes.

## Discussion



Recent advances in analyses techniques have shown that the spike timing model (firing rate, interspike interval) is incomplete in terms of describing neuronal activity and information transfer in neurons<sup>16,17</sup>. Using the tetrode system as a frame of reference, we have shown that an analysis of the newly described feature of spike directivity has important implications to understanding neuronal activity<sup>16,19</sup>. Based on electrical recordings and computational methods we are able to show that in certain neurons, spike directivity changes occur in a determined, behaviorally dependent fashion with events on the T-maze. Spike directivity is a hidden characteristic. Unlike spike timing, directivity cannot be directly perceived by a human observer, it has to be computed in advance to allow further analysis. Additionally, as spike counts may decrease with learning, firing rate dependent analyses and ISI statistics cannot be performed. Since spike directivity is computed for every spike, only few spikes are required to expose the significance of this feature. Coupled with spike timing analysis, our work uses spike directivity to reveal a hitherto unrecognized yet important aspect of how behavior is expressed by neurons.

The first result is that “expert” units responded to more than one meaningful event. This suggests that spiking behavior of each cell is likely to represent more than one class of events and does not imply the existence of single neurons coding uniquely for certain discrete events. This is seen in the temporal response pattern in expert neurons (Fig 2a and b) and equally well demonstrated in the directivity analysis of spikes from the same neurons (Fig 3a and b) confirming the published observations that neurons do respond by changing spiking activity in specific periods of the T-maze behavior. The changes in the cosines angle in spike directivity have similar inflections as spike activity plots, corresponding to the same phases in the same neuron. Therefore, alterations in spatial directivity occur simultaneously to changes in the spike timing.

It is likely that immediately after delivery of the auditory tone, action selection of which turn is to be taken occurs, which is then followed by turn execution. Neurons that respond to meaningful events of the task appear to have robust firing rate changes to periods corresponding to “before” and “during” the actual turn. This implies that action selection and execution can be represented in the same expert neuron as shown in both the temporal and spike directivity analyses. Such neurons can then be seen to combine spatial directivity of spiking with time contingent properties.

Yet a crucial difference exists between what we gain from spike time versus directivity analysis. If the spiking activity is considered, one can see clearly the occurrence of turn selection and execution in a successive manner. However, based on estimated spike activity (Figure 2a and b, Supplementary Figs 1 c and d), an observer would be unlikely to determine the direction of the turn that the animal has selected since there is no

significant difference between temporal spike activity corresponding to each direction of turn. This crucial piece of information that assigns which turn selection is valuable, i.e. has the potential to result in reward is however elegantly revealed in the spike directivity characteristic. Our previous work demonstrated that spike directivity organizes with learning<sup>16</sup>. The current result demonstrates that this organization during the period between tone and beginning of turn indicates the arm to be selected, information which is of great value to the animal in order to obtain reward. Most importantly, this separation in spike directivity does not occur in the previous phase, between gate opens and tone delivery (Fig 3b) and is not present after the selection of turn between the turn starts and goal is achieved (Fig 3a).

The conclusion is then obvious that upon tone delivery, the spike directivity that separates the left versus the right turn represents the selection phase. Since correct selection increases the chances of reward, this intriguing result then implies that the spike directivity predicts which turn is subsequently to be executed.

During this action selection phase, the number of spikes decreases with learning. However, the spike directivity analysis shows that each spike now has more value in indicating the turn arm being selected. It is intriguing to suggest that the occurring spikes are now more efficient at providing information about the task. The cosine between two spike directivity vectors from the same neuron or just their scalar product can be considered to measure the semantic distance between neuronal spikes. The computed semantic distance of spikes (see Supplementary Methods) shows the similarity of meaning between two spikes from the same neuron implying that the more separated the directivity between spikes, the more distinct the spikes are semantically. Indeed one may predict the animals' turn direction on the T-maze simply based upon the directivity of spikes in the selection period. Once organized, at a point when the animal has acquired the task, the rat "understands" the meaning of the low or high tone and selects the turning arm to maximize the way of getting the reward.

Summarizing then, this paper shows that the same "expert" neurons respond to multiple events of the T-maze task, that they show response modulations in spiking frequency (temporal domain) and in spike directivity (spatial domain). This temporal and spatial responsivity occurs to similar events in the task. In addition, we show that spike directivity can be correlated with meaning in terms of which turn selection would have the highest likelihood of obtaining reward. We anticipate that these changes in spike directivity could be a manifestation of a form of neuronal plasticity. Since electrical flux of charges carry information<sup>17</sup>, "expert" neurons can be seen as projecting information

related to behavior, temporally and spatially, to different targets at certain moments in time. The directionality of charge flow as observed by the tetrodes is a reflection of intracellular and dendritic events that may to some extent be determined by the received inputs<sup>16,19</sup>.

Communication in the brain appears to be represented by such spatiotemporal electrical flux on a small scale which contains behaviorally meaningful data that is transferred between anatomically interconnected structures. Such electrical waves can be effectively measured in the brain in recordings such as field potentials or electroencephalography and identified as rhythms of the brain<sup>28</sup>. In our work, the use of tetrodes provides a local frame of reference that allows the visualization of these subtle changes in time and space on a small spike-level scale.

Finally and most importantly, our work has shown that predictability of the upcoming behavior is an inherent property of analysed neurons as demonstrated by their spatio-temporal dynamics. Based on measured electrical activity from tetrode recordings, the demonstration of such subtle yet necessary feature is in essence equivalent to reading the neural code.

## **Materials and methods**

We analyzed electrical activity of isolated units from the dorsolateral portion of the striatum of 3 Sprague-Dawley rats (Supplementary Methods). Tetrode recordings were obtained with established methods<sup>10,27,29</sup> and data was captured at an acquisition rate of about 25 KHz per channel using a Neuralynx@ data acquisition system. On average, 6 tetrodes were available for analysis in each animal. Subsequent processing included clustering into putative neurons and de-noising of the data (Supplementary Methods). The details of spike activity estimation (Supplementary Methods), spike directivity computation based on the charge movement model are presented in<sup>15</sup> and also briefly explained in Supplementary Methods.

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