Integration of visual motion and locomotion in mouse visual cortex

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Successful navigation through the world requires accurate estimation of one’s own speed. To derive this estimate, animals integrate visual speed gauged from optic flow and run speed gauged from proprioceptive and locomotor systems. The primary visual cortex (V1) carries signals related to visual speed, and its responses are also affected by run speed. To study how V1 combines these signals during navigation, we recorded from mice that traversed a virtual environment. Nearly half of the V1 neurons were reliably driven by combinations of visual speed and run speed. These neurons performed a weighted sum of the two speeds. The weights were diverse across neurons, and typically positive. As a population, V1 neurons predicted a linear combination of visual and run speeds better than either visual or run speeds alone. These data indicate that V1 in the mouse participates in a multimodal processing system that integrates visual motion and locomotion during navigation.

There is increasing evidence that activity in area V1 is determined not only by the patterns of light falling on the retina, but also by multiple nonvisual factors. These factors include sensory input from other modalities, the allocation of spatial attention and the likelihood of an impending reward. In mice, in particular, firing of V1 neurons shows strong changes with locomotion, but the precise form and function of this modulation are unclear. One possibility is that locomotion simply changes the gain of V1 neurons, increasing their responses to visual stimuli when animals are running compared to when they are stationary. Another possibility is that V1 neurons respond to the mismatch between what the animal sees and what is expected based on locomotion. Overall, the computational function of locomotor inputs to V1 is still a mystery. Might they be useful for navigation?

One of the primary roles of vision is to help animals navigate. Successful navigation requires an accurate estimate of one’s own speed. To obtain this estimate, animals and humans integrate a measure of speed gauged from optic flow with one gauged from the proprioceptive and locomotor systems. Neural correlates of locomotor speed have been found in high-level structures such as the hippocampus, but the neural substrates in which multiple input streams are integrated to produce speed estimates are unknown.

Integration of multiple inputs has been observed in several neural circuits and typically involves mixed neuronal representations. In early processing, the input streams are integrated into a distributed population code, in which the input signals are weighted differently by different neurons. Such a mixed representation allows the integrated signal to be read out by higher-level structures. Properties of this mixed representation (such as the statistical distribution of weights used by different neurons) are not random but adapted to the specific integration that must be performed.

Here we studied how visual and locomotion signals are combined in mouse V1, using a virtual reality system in which visual input was either controlled by locomotion or was independent of locomotion. We found that most V1 neurons responded to locomotion even in the dark. The dependence of these responses on running speed was gradual, and in many cells it was non-monotonic. In the presence of visual inputs, most V1 neurons that were responsive encoded a weighted sum of visual motion and locomotor signals. The weights differed across neurons and were typically positive. As a population, V1 neurons encoded positively weighted averages of speed derived from visual and locomotor inputs. We suggest that such a representation facilitates computations of self motion, contributing to the estimation of an animal’s speed through the world.

RESULTS

To study the effects of visual motion and locomotion, we recorded from mouse V1 neurons in a virtual environment based on an air-suspended spherical treadmill. The virtual environment was a corridor whose walls, ceiling and floor were adorned with a white noise pattern. We presented four prominent landmarks (three gratings and a plaid) at equal distances along the corridor. Head-fixed mice viewed this environment on three computer monitors arranged to cover 170° of visual angle. While the mice traversed this environment, we recorded from populations of V1 neurons with multisite electrodes and identified the spike trains of single neurons and multiunit activity with a semiautomatic spike-sorting algorithm. We then used grating stimuli to measure the receptive fields of the neurons (receptive field size of 24° ± 2° (±s.e.), n = 81 neurons, receptive field centers with 10°–70°...
azimuth, \( n = 84 \) neurons, semisaturation contrast of 23 ± 3% (±s.e.), \( n = 38 \) neurons).

To measure the features that influenced neural activity in the virtual environment, we adopted a technique previously used for analysis of hippocampal place cells.\(^{29,30}\) For each neuron, we estimated the firing rate as a function of one or more predictor variables (for example, speed). Using a separate data segment (the ‘test set’), we defined a prediction quality measure \( Q \) as the fraction of the variance of the firing rate explained by the predictor variables. This measure of prediction quality does not require that stimuli be presented multiple times (Supplementary Fig. 3), a key advantage when analyzing activity obtained during self-generated behavior.

We first measured responses of V1 neurons in a closed-loop condition, where the virtual environment was yoked to running speed and thus faithfully reflected the movement of the animal in the forward direction. For each neuron, we computed our ability to predict the firing rate based on position alone \( (Q_0) \), on speed alone \( (Q_S) \), and on both position and speed \( (Q_{PS}) \). The responses of most V1 neurons (181/194 neurons) were predictable based on both the position and speed in the environment \( (Q_{PS} > 0); \) Supplementary Fig. 5). Here we concentrate on 110 of these neurons whose firing rates were highly repeatable \( (Q_{PS} > 0.1). \) Some of these neurons \( (51/110 \) neurons) showed clear modulation in firing rate as a function of position \( (Q_0 > 0.1). \) We expected to observe this tuning for position because of the different visual features present along the corridor, and indeed neurons often responded most strongly as the animal passed the visual landmarks (Fig. 1e and Supplementary Fig. 5). The response of most neurons \( (81/110 \) neurons) also showed a clear dependence on speed \( (Q_S > 0.1). \) Typically, speed explained a larger fraction of the response than position did \( (75/110 \) neurons with \( Q_S > Q_0; \) Fig. 1f,g). Indeed, for many neurons the responses were as well predicted by speed alone as by speed and position together \( (Q_S/Q_{PS} \approx 1; \) Fig. 1g and Supplementary Fig. 5). Speed, therefore, exerts a powerful influence on the responses of most V1 neurons in the virtual environment.

But what kind of speed is exerting this influence? In virtual reality, the speed at which the virtual environment moves past the animal (‘virtual speed’) is identical to the speed with which the animal runs on the air-suspended ball (‘run speed’). Neurons in V1 can gauge virtual speed through visual inputs, but to gauge run speed they must rely on nonvisual inputs. These could include sensory input from proprioception, and top-down inputs such as efference copy from the motor systems. Ordinarily, virtual and run speeds are identical, so their effects on V1 cannot be distinguished.

One way to isolate run speed from virtual speed is simply to occlude the visual input. To do this, we turned off the monitors and occluded all other sources of light, and thus measured responses to run speed in the dark. Running influenced the activity of many V1 neurons even in this dark environment \( (39/55 \) well-isolated neurons were significantly modulated, \( P < 0.001, \) sign-rank test), modulating their activity by 50% to 200% (Supplementary Fig. 6). The dependence of firing rate on run speed was graded, rather than a simple binary switch between different rates in stationary and running periods (Fig. 2a–c). Among the neurons modulated by running, most \( (27/39 \) neurons) showed a significant \( (P < 0.001, \) sign-rank test) dependence on run speed even when we excluded stationary periods \( (run \ speed < 1 \ cm/s). \) About half of these neurons \( (16/27 \) neurons) showed a band-pass tuning characteristic, responding maximally to a particular run speed \( (Fig. 2b,d \text{ and Supplementary Fig. 7}); \) in the rest, firing rate showed either a monotonic increase with run speed \( (Fig. 2a; 7/27 \) neurons) or a monotonic decrease with run speed \( (Fig. 2c, 4/27 \) neurons).
Figure 2 Tuning of V1 neurons for run speed in the dark. (a–c) Dependence of firing rate on run speed for three V1 neurons measured in the dark. Error bars, s.e., n > 1,800 time bins (16.7 ms each). Sampling bins were spaced to have equal numbers of data points; curves are fits of a descriptive function (Online Methods). Arrows indicate the speed at the maximal response and open circles the firing rates when the animal was stationary. d31, d113 and d158 indicate the neuron identity. (d) Preferred run speed (peak of the best-fit curve) for neurons that showed a significant (P < 0.001, sign-rank test) nonbinary modulation of firing rate as a function of run speed (n = 27 well-isolated neurons). Neurons where the preferred speed was < 2 cm s−1 were considered low-pass (low; example in c); neurons where the preferred speed was > 25 cm s−1 were considered high-pass (high; example in a), and the remainder were considered bandpass (example in b).

We obtained similar results when the monitors were turned on but displayed a uniform gray screen (data not shown). Thus, the responses of V1 neurons depend smoothly and in diverse ways on the speed at which the animal runs, even in the absence of visual inputs.

To understand how run speed affects V1 responses to visual stimuli, we reconfigured the virtual reality environment into an open-loop condition. In this condition we simply replayed movies of previous closed-loop runs, irrespective of the animal’s current run speed. Whereas in the closed-loop condition virtual speed and run speed were always equal, in the open-loop condition the animal experienced the virtual environment at different combinations of virtual and run speeds (Fig. 3a). We could investigate the influence of both speeds because the mice did not attempt to adjust their running based on visual inputs: there was little correlation between run speed and virtual speed (r = 0.07 ± 0.05 (± s.e.)). Similarly, V1 neurons did not modify their responses in the two conditions: when the two speeds happened to match during the open-loop condition, responses of V1 neurons were similar to those measured in the closed-loop condition.

Figure 3 V1 neurons are tuned for a weighted sum of run speed and virtual speed. (a) Some paths in virtual speed and run speed taken by an animal in the open-loop condition (representative example of the 11 experimental sessions). (b–d) ‘Speed maps’ showing firing rate of three example neurons (representative of 73 neurons with QRV > 0.1) as a function of virtual speed and run speed (bottom left); dependence of firing rate on run speed alone (bottom right); and dependence of firing rate on virtual speed alone (top left). Numbers to the right of color bars indicate neuron identity. Error bars, s.d. over n = 10 training sets. (e) In the weighted-sum model, firing rate is a nonlinear function f of a weighted sum of virtual speed V and run speed R. The weights α and β are summarized by a single interaction angle θ = tan−1(α/β). (f–h) Predictions of the full-speed maps by the model (left; compare to b–d). Color scales same as for corresponding neurons in b–d. The model’s predictive power as a function of θ (right). Optimal interaction angle θmax is highlighted in red and indicated as a vector on the left. Dashed line represents the predictive power of the original speed map. Color map is the same as for corresponding neurons in b–d. (i) Comparison of predictive power using the weighted sum model (QRV) at the optimal interaction angle (θmax), to that using the speed map (QRV). Dashed line indicates equal performance. Blue points mark the examples in f–h. Solid dots indicate well-isolated units. (j) Distribution of optimal interaction angles θmax across neurons. Black bars indicate the distribution for well-isolated units.
Figure 4 V1 neuron population activity encodes positive combinations of virtual and run speeds. (a) Activity of 24 V1 neurons during an epoch of open-loop navigation. Firing rate of each neuron was normalized to range from 0 to 1 for illustration purposes. (b) Prediction of the linear combinations of run speed and virtual speed based on the activity of a population of V1 neurons, using a linear decoder that was trained and evaluated on separate parts of the data set. The black curve shows a weighted average of virtual speed and run speed ($\theta = 60^\circ$), and the red curve its prediction from population activity, for the same epoch as in a. (c) Performance of the population decoder as a function of $\theta$, for a single experimental session. Error bars, s.e. across $n = 5$ runs of cross-validation. Dashed lines, mean performance across all interaction angles. The circled point indicates the example shown in b. Gray, performance when the decoding is restricted to periods when both virtual speed and run speed were $>3$ cm s$^{-1}$. (d) Decoding performance as a function of $\theta$ across recording sessions. Error bars, s.e. across sessions ($n = 11$ (red) and $n = 9$ (gray)).

(Supplementary Fig. 8). We therefore used responses from the open-loop condition to measure the effects and interactions of the two speeds.

In the open-loop condition, responses of V1 neurons were modulated by both run speed and virtual speed. Some neurons were strongly influenced by virtual speed (Fig. 3c–d: 28/173 neurons with $Q_V > 0.1$); this was expected because translation of the virtual corridor causes strong visual motion across the retina, and visual motion is a prime determinant of responses of V1 neurons. The responses of many neurons were also modulated by run speed (39/173 neurons with $Q_R > 0.1$; Fig. 3b–d). This modulation was not due to eye movements (Supplementary Fig. 9). As in the absence of visual stimuli, responses varied smoothly with run speed. Indeed, firing rates were better predicted by a smooth function of speed than by a binary function with one value each for the stationary and running conditions ($Q_{\text{binary}} < Q_R$; $P < 10^{-8}$, sign-rank test).

There was no obvious relationship, however, between tuning for virtual speed and for run speed (Supplementary Fig. 10); rather, the firing of most neurons depended on the two speeds in combination, with different cells performing different combinations. To study these combinations, for each neuron we derived a ‘speed map’, an optimally weighted sum of the two speeds. This model requires a single parameter $\theta$, the ‘interaction angle’ determined by the two weights, and a binary $< 1$ for illustration purposes. ($Q_{\text{binary}} < Q_R$; $P < 10^{-8}$, sign-rank test).

To study the relative weighting of virtual speed and run speed, we adopted a simple model based on a weighted sum of the two speeds. This model requires a single parameter $\theta$, the ‘interaction angle’ determined by the two weights, and a binary $< 1$ for illustration purposes. ($Q_{\text{binary}} < Q_R$; $P < 10^{-8}$, sign-rank test).

To summarize and quantify how a neuron’s firing rate depends on the open-loop condition to measure the effects and interactions of the two speeds.

To study the relative weighting of virtual speed and run speed, we adopted a simple model based on a weighted sum of the two speeds. This model requires a single parameter $\theta$, the ‘interaction angle’ determined by the two weights, and a binary $< 1$ for illustration purposes. ($Q_{\text{binary}} < Q_R$; $P < 10^{-8}$, sign-rank test).
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rely on responses measured during stationary conditions. To do this, we restricted the decoding to epochs when both run speed and virtual speed were > 3 cm/s, and we found a similar dependence of decoding performance on interaction angle (Fig. 4c, d; circular mean = 42°; circular-linear correlation, P < 0.01; the reduction in decoding performance in the restricted analysis suggests that the population encodes both smooth changes of speed and binary changes: stationary versus moving). Second, we ensured that these results did not depend on the precise choice of decoding algorithm. We observed the same dependence of decoding performance on interaction angle with two alternate decoders (Supplementary Fig. 11). Finally, we asked whether these results reflect the distribution of optimal interaction angles that we measured. We used simulations of V1 populations with different distributions of interaction angles (Supplementary Fig. 12). We could replicate the profile of decoding performance present in the data only when the distribution of interaction angles in the simulated population resembled that of the real population (Supplementary Fig. 12c–j).

The population of V1 neurons therefore encodes positively weighted linear combinations of run speed and virtual speed more accurately than virtual speed or run speed alone.

DISCUSSION

Measuring activity in visual cortex in a virtual reality environment revealed a number of interactions between visual motion and locomotion. First, we replicated the observation that running affects V1 responses6,7, even in the absence of visual stimuli, and we extended it by showing that these responses vary smoothly and often non-monotonically with run speed. Further, we found that V1 neurons typically respond to combinations of run speed (gauged from locomotor and proprioceptive systems) and visual speed (gauged from optic flow). This combination of run speed and visual speed is simply a weighted sum, with weights varying across neurons. Most neurons gave positive weights to visual and run speeds (0° ≤ θ < 90°). Accordingly, the population of V1 neurons was most informative as to positively weighted linear combinations of run and visual speeds.

The fact that V1 integrates locomotor and visual signals in this manner suggests that it may be an early stage of a pathway for estimating an animal’s speed through the world, which can then help functions such as navigation. However, two alternate hypotheses have also been suggested for the presence of run speed signals in V1.

A first alternate hypothesis is that locomotion simply changes the gain of sensory responses of V1 neurons without affecting the selectivity of visual neurons. In support of this hypothesis are observations that locomotion scales, but does not modify, the visual preferences of V1 neurons6. Our data are not fully consistent with this interpretation for multiple reasons. First, we and others7, find responses to run speed even in the absence of visual stimuli, suggesting that locomotor signals provide a drive to V1 neurons and not just a modulation of visual responses. Indeed, there is evidence that locomotion alters the visual preferences of V1 neurons particularly preferred stimulus size6. We also found that responses to running were different across neurons, inconsistent with modulation of an entire visual representation by a single locomotor-dependent gain function. Previous data suggested that the effect of locomotion was binary6, as would be expected if running caused a discrete change in cortical state31. However, we found that a binary model did not predict the firing-rate responses as well as a continuous dependence on firing rate. Our data therefore indicate that locomotor effect on the responses of V1 neurons go well beyond a uniform difference in gain between running and stationary animals.

A second alternate hypothesis holds that V1 signals the mismatch between actual visual stimuli and those that should have been encountered given the locomotion. This explanation fits the theoretical framework of predictive coding32 and is supported by a recent report using two-photon imaging of superficial V1 neurons7. By exploring all combinations of run speed and visual speed, however, we found that only a small minority of V1 neurons (5/73 neurons) were selective for mismatch. Perhaps this discrepancy results from different selection biases in the two recording methods: whereas our silicon probe recordings primarily recorded neurons from the deeper layers of cortex, two-photon imaging reports only neurons from layer 2/3; the possibility that prediction errors are specifically encoded in superficial layers has in fact been suggested by computational models33. However, a more likely reason may be differences in stimulus design. We avoided sudden perturbations of the visual stimulus, whereas the previous study7 specifically focused on such sudden perturbations. Such perturbations may trigger a change in alertness and often evoked behavioral response (slowing down) in that study7. Behavior can evoke calcium responses in the sensory cortex34,35, making it hard to disambiguate the influence of sensory mismatch from its behavioral consequences. Thus, the lack of sudden perturbations of the visual stimulus in our experiments might explain the differences in the observations.

The circuit mechanisms underlying the effects we described are most likely the same ones that support the effects of locomotion on spatial integration: locomotion can affect size-tuning, preferentially enhancing responses to larger stimuli6. This finding is compatible with many of our current results, but is not sufficient to predict them; for example, the tuning for run speed that we observed here in the dark certainly could not be predicted by changes in spatial integration. The effects of locomotion may be caused by neuromodulators such as norepinephrine46. Our data are not inconsistent with this possibility, although the smooth (and sometimes band-pass) modulation of firing with running speed would require the neuromodulatory signal to encode speed in an analog manner. Furthermore, the diverse effects of running we observed across neurons would suggest that a diverse prevalence of receptors or circuit connections underlie the tuning for run speed.

In our experiments we recorded from animals that navigated a familiar environment, in which the distance between the animal and the virtual wall (equivalently, the gain of the virtual reality system) was held constant. The mice had experienced at least three training sessions in closed-loop mode before recording, which would be sufficient for the hippocampus to form a clear representation of the virtual environment15 and presumably would be sufficient for the animal to learn the stable mapping between movements and visual flow. In a natural environment, however, an animal’s distance to environmental landmarks can rapidly change. Such changes can lead to rapid alteration in visuomotor gain, accompanied by changes in neural activity at multiple levels, as demonstrated, for instance, in zebrafish37. Furthermore, both animal behavior and neural representations can adjust to the relative noise levels of different input streams, in a manner reminiscent of Bayes optimal inference19,38. In the case of mouse navigation, such changes should cause a reweighting of run and visual speeds in the estimation of an animal’s own running velocity. Such a reweighting could occur through alteration of the V1 representation, by changing the distribution of weights of visual and running speeds to center around a new optimal value. Alternatively, however, such changes could occur outside of V1. The latter possibility is supported by the fact that the representation we observed in V1 allows readouts of a wider range of run–visual mixtures than a simulated population in
which all neurons encoded a single interaction angle (Supplementary Fig. 12). Additional experiments will be required to distinguish these possibilities. We also note that, although head-fixed animals are certainly capable of navigation in virtual reality, animals that are not head-fixed gain an important cue for speed estimation from the vestibular system. To understand how this vestibular signal affects integration of visual and locomotor information one should record from V1 neurons in freely moving animals.

Our results suggest that the function of mouse visual cortex may be more than just vision. A growing body of evidence suggests that neocortical areas are not specific to a single function and that neurons of even primary sensory cortices can respond to a wide range of multimodal stimuli and nonsensory features. Our results provide a notable example of such integration and suggest an ethological benefit it may provide to the animal. Estimation of speed through the world is a critical function for navigation and is achieved by integrating inputs from the visual system with locomotor variables. Our data indicate that, at least in the mouse, this integration occurs as early as in V1.

METHODS

Methods and any associated references are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

All the authors contributed to the design of the study and to the interpretation of the data. A.B.S. and A.A. carried out the experiments, A.B.S. analyzed the data, and A.B.S., M.C. and K.D.H. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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Surgical planning. Five wild-type mice (C57BL6, 4–9 week-old males; 20–26 g) were chronically implanted with a custom-built head post and recording chamber (4 mm inner diameter) under isoflurane anesthesia. No statistical methods were used to predetermine group sizes; the sample sizes we chose are similar to those used in previous studies. We did not require blinding and randomization as only wild-type mice were used. On subsequent days, implanted mice were acclimatized to run in the virtual environment in 20–30 min sessions (4–12 sessions), until they freely ran 20 traversals of the environment in 6 min.

One day before the first recording session, animals were anesthetized under isoflurane and a ~1 cm craniotomy was performed over area V1 (centered at 2.5 mm lateral from midline and 0.5 mm anterior from lambda). The chamber was then sealed using silicone elastomer (Kwik-Cast).

Recordings. To record from V1 we inserted 16-channel linear multisite probes (with site of size 312 or 430 μm, spaced 50 μm apart, NeuroNexus Tech.) spanning depths of 100–850 μm. Recordings were filtered (0.3–5 KHz), threshold crossings were auto clustered using Klustawik28 followed by manual adjustment using Klusters27. A total of 194 units were isolated, of which 123 were in deep layers (channel >10, numbered surface to ‘deep’), 11 in superficial layers (channel < 6; we found layer 4 to be located around channels 6–10 based on a current source density analysis). 46 isolated units were judged to be well-isolated (isolation distance48 >20). All examples shown in this paper are well-isolated units. The analysis of data included all units (except dark condition), as restricting the analysis of the data to only well-isolated units did not affect the results. There was no correlation between spike isolation quality and Qp (p = 0.06) or Qps (p = 0.07). The firing rate of each unit was calculated by smoothing its spike train using a 150-ms Gaussian window. All the stimuli and spiking activity were then sampled at 60 Hz, the refresh rate of the monitors.

Virtual environment. Visual scenes of a virtual environment were presented on three monitors (19-inch LCD, HA191, Hanns.G, mean luminance 50 cd/m², 35 cm away from the eye) that covered a visual angle of 170° azimuth and 45° elevation. Mice explored this environment by walking over an air-suspended spherical treadmill49 (Fig. 1a).

The environment was simulated in Matlab using the Psychophysics toolbox50,81. The virtual environment was a corridor (120 cm × 8 cm × 8 cm) whose walls, ceiling and floor were adorned all along the corridor with a filtered white noise pattern of full Michelson contrast (overall root mean square (RMS) contrast: 0.14), and four positions in the corridor had prominent patterns as landmarks: gratings (oriented vertical or horizontal of full Michelson contrast, RMS contrast: 0.35) in three positions and a plaid (overlapping half-contrast horizontal and vertical gratings, RMS contrast: 0.35) in the fourth (Fig. 1a,b). Movement in the virtual reality was constrained to one-dimensional translation along the length of the room (the other two degrees of freedom were ignored). All speeds < 1 cm/s were combined into a single bin unless otherwise specified. The gratings had a spatial wavelength of 1 cm on the wall, which is equivalent to a spatial frequency of 0.09 cycles/cm at a visual angle of 45° azimuth and 0° elevation. The white noise pattern was low-pass Gaussian filtered with a cutoff frequency of 0.5 cycles/cm2 at 45° azimuth. Owing to the three-dimensional nature of the stimuli, the spatial frequency (in cycles/s) and visual speed (in °/s) presented are a function of the visual angle. Therefore, the speed of the visual environment is defined in terms of the speed of movement through the virtual-reality environment, the virtual reality speed (virtual speed in cm/s). In the closed-loop condition, this is the speed matched to what the animal would see if it were running in a real environment of the same dimensions. For reference, at a visual angle of 60° azimuth and 0° elevation, a virtual speed of 1 cm/s corresponds to a visual speed of 9.6°/s, 10 cm/s to 96°/s and 30 cm/s to 288°/s. The running speed of the animal is calculated based on the movement of the air-suspended ball in the forward direction, as captured by the optical mice49.

Mice first ran the closed-loop condition (>20 runs through the corridor), followed by two sessions of the open-loop condition. On reaching the end of the corridor on each run, the animals were returned (virtually) to the start, after a 3-s period during which no visual stimuli were presented (gray screen). In the open-loop condition, movies generated in closed-loop were simply played back, regardless of the animal’s run speed. For three animals (6 sessions), the closed-loop condition was repeated after the open-loop sessions. After the measurements in virtual reality, we mapped receptive fields using traditional bar and grating stimuli. Each animal was taken through 1–3 such recording sessions.

Response function. The response of each neuron (shown in Figs. 1 and 3) was calculated as a function of the variables of the virtual environment and their various combinations using a local smoothing method previously used to compute hippocampal place fields52–54. For example, a neuron’s firing rate ρ(t), at time t, was modeled as a function of χ(a(t)) over the variable a. To estimate the model χa, the variable a was first smoothed in time (150 ms Gaussian) and discretized in n bins to take values a1, a2,..., an (the number of bins n was taken as 150 for position, 30 for speeds; the precise bin numbers were not important as response functions were smoothed). We then calculated the spike-count map S and occupancy map Φ. Each point of the spike-count map was the total number of spikes when a(t) had a value of ai: Si = ∑t ρ(t(ai))δ(t), where δ is the index of bins of variable a. The occupancy map was the total time spent when the variable a had a value of a: Φ = ∑t ρ(t(ai))δt, where δt was the size of each time bin (δt = 16.67 ms). Both S and Φ maps were smoothed by convolving them with a common Gaussian window whose width σ (ranging between 1 bin to total number of bins) was optimized to maximize the cross-validated prediction quality (see below). The stationary (run speed ≤ 1 cm/s) or static (virtual speed ≤ 1 cm/s) bins were not included in the smoothing process. The firing rate model was then calculated as the ratio of the smoothed spike count and occupancy maps:

\[
\chi_a = \frac{S_i}{Φ_i(a)} \cdot (0,\sigma)
\]

where η0(0, σ) is a Gaussian of width σ and the operator ‘*’ indicates a convolution. Based on the model, fit over training data, we predicted the firing rate over the test data as

\[
y^*(t) = \chi_a(a(t))\]

where y*(t) is the prediction of firing rate based on variable a. A similar procedure was followed for the two-dimensional ‘speed maps’, where two independent Gaussians were used to smooth across each variable.

Prediction quality. Response functions and models were fit based on 80% of the data and the performance of each of models was tested on the remaining 20% (cross-validation). We calculated the prediction quality Qa of model χa based on variable a, as the fraction of variance explained:

\[
Q_a = 1 - \frac{\sum_i (y(t) - y^*(t))^2}{\sum_i (y(t) - \mu)^2}
\]

where y(t) is the smoothed firing rate (150 ms Gaussian window) of the neuron at time t, y*(t) was the prediction by model χa for the same time bin and μ is the mean firing rate of the training data. A value of Q close to 0 suggests the model does not predict the firing rate of the neuron any better than using a constant and a higher Q suggests good performance. Values of Q close to 1 are unlikely as the intrinsic variability in the response of neurons, which is uncorrelated to the stimulus, is not captured by any of the models. Very low values of Q suggest that the response is unreliable. Therefore, we only concentrated on neurons whose responses were reliable for further analysis by setting a limit of Q > 0.1 (Fig. 1g: 110/194 neurons with Qps > 0.1 and Fig. 2j: 73/194 neurons with Qmap > 0.1). To compare the metric explained variance of the mean response which is more commonly used, we calculated Q and explained variance on direction tuning of neurons. We found that neurons with a model for direction tuning of Q > 0.1 had an explained variance in the range of 0.75–0.97 (two examples are shown in Supplementary Fig. 3). To test the alternative hypothesis of a binary model of run speed, we only considered two bins, which were whether the animal ran (speed > 1 cm/s) or not. We used the mean firing rate of the training data in these bins, to predict firing rate of the test data and calculate Qbinary.

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Responses in darkness. We trained a separate set of animals (n = 3 mice) with an additional 5–10 min condition of complete darkness. The darkness was achieved by turning all the non-essential lights and equipment in the room off. Light from essential equipment outside the recording area (for example, recording amplifiers) was covered with a red filter (787 Marius Red; LEE filters), rendering any stray light invisible to mice. As a result, the recording area was completely dark both for mice and for humans (luminance <10⁻² cd/m², i.e., below the limit of the light meter). We used 32-channel, 4-shank multisite probes (spaced 200 μm apart) to record one session on each animal; each shank had two sets of tetrodos spaced 150 μm apart (each electrode had recording sites of 121 μm², Neuronexus). We recorded a total of 145 units of which 55 were well-isolated units (isolation distance > 20). We only considered the well-isolated units for analysis in the dark condition. Similar results were obtained when considering all units (data not shown). The dark condition during the three recording sessions lasted 8 min, 9 min and 13 min. In this condition, speed was defined as the amplitude of the two-dimensional velocity vector.

Responses in the dark condition were calculated by discretizing the run speed such that each speed bin contained at least 7% of the dark condition (>30 s). In cases where the animal was stationary for long periods of time (>7%), the stationary speed (≤1 cm/s) bin had more data points. We calculated the mean and error of the firing rate in each of the speed bins (Fig. 2). The speed in any bin was the mean speed during the time spent at that speed bin. To assess statistical significance of modulation by run speed, for each neuron we recalculated the firing rate as a function of speed in each bin after shuffling the spike times. As a conservative estimate, we considered a neuron’s response to be significantly modulated by run speed if the variance of its responses was greater than 99.9% of the variance of the its shuffled responses (P<0.001). To test whether the neuron’s response was significantly nonbinary, we followed the same procedure as above, but restricting the test to only periods when run speed was >1 cm/s.

To characterize the run speed responses we fit the mean responses to speed s (s > 1 cm/s) by the following descriptive function\(^{53,56}\),

\[
y(s) = y_{\text{max}} \exp\left\{- (s - s_{\text{max}})^2 / \sigma(s) \right\}
\]

where \(\sigma(s)\) was \(\sigma_{\text{r}}\) if \(x < s_{\text{max}}\) and \(\sigma_{\text{r}}\) if \(x > s_{\text{max}}\) and \(y_{\text{max}}, s_{\text{max}}, \sigma_{\text{r}}\) and \(\sigma_{\text{r}}\) were the free parameters. We fit three curves by adding constraints on \(s_{\text{max}}\): (i) a monotonically increasing function was fit by constraining \(s_{\text{max}}\) to be greater than \(\leq 30 \text{ cm/s}\), (ii) a monotonically decreasing function was fit by constraining \(s_{\text{max}}\) to be \(\leq 1 \text{ cm/s}\), and (iii) a bandpass curve by not constraining \(s_{\text{max}}\). These three curves were fit on 80% of the data, and we tested the fraction of explained variance of the firing rate on the remaining 20%. We considered a neuron bandpass only if the variance explained by the band-pass curve was greater than both a monotonically increasing or decreasing curve and when \(s_{\text{max}}\) was > 2 cm/s and < 25 cm/s.

Weighted sum model. The firing rate of a neuron \(i\) at time \(t\), \(y_i(t)\) was modeled as

\[
y_i(t) = f(\alpha V(t) + \beta R(t))
\]

where \(\alpha = \sin(\theta), \beta = \cos(\theta), V(t)\) is the virtual speed and \(R(t)\) is the run speed of the animal at time \(t\). The function \(f\) was estimated using a one-dimensional version of the same binning and smoothing procedure described above for estimating response functions. For each cell, the model was fitted for a range of integration angles \(\theta\) from 0° to 180° in 16 steps. The optimal integration angle \(\theta_{\text{max}}\) was chosen as the value of \(\theta\) giving the highest cross-validated prediction quality.

Population decoding. The smoothed (0.9 s Gaussian window) firing rate vector of all simultaneously recorded neurons was used to predict a linear combination of virtual speed and run speed \(\sin(\theta) V(t) + \cos(\theta) R(t)\) for a range of integration angles \(\theta\) from 0° to 180° in eight steps. As our hypothesis is to test the decoding of speed relevant to navigation, we consider all speeds <3 cm/s to be stationary (Fig. 4b–d), or ignored all the times when either run speed or visual speed were <3 cm/s (Fig. 4c,d: we only considered sessions (9/11) where >100 s fulfilled this criterion). Reducing the limit to <1 cm/s or 0.03 cm/s did not affect the trend in the decoding performance (Supplementary Figs. 11 and 12). We used a linear decoder (ridge regression, with the ridge parameter optimized by cross-validation), to evaluate how well an observer could decode a combination of speeds given the firing rate of the population. The performance of the decoder was tested as the fraction of variance explained on an independent 20% of the data that was not used to train the decoder.

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