Supplemental Information

Opposing Influence of Top-down and Bottom-up

Input on Excitatory Layer 2/3 Neurons

in Mouse Primary Visual Cortex

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Cell group	Inclusion criteria	Sample sizes
L2/3 putative excitatory neurons	Spike half-width > 0.6 ms, R_{input} < 100 MΩ, vertical depth < 400 µm	total: 32 (dMM: 17, hMM: 6, unclassified: 9)
L5/6 putative excitatory neurons	Spike half-width > 0.6 ms, R_{input} < 100 MΩ, vertical depth > 480 µm	total: 14
Figure panels		
2B, 2D, 6E, 6F, S6A, S6B, S3C, S3D, S5D 3A (middle and bottom plot)	No inclusion criteria applied	L2/3: 32 (dMM: 17, hMM: 6, unclassified: 9) L5/6: 14
2G, S3H, S6D	# Mismatch trials ≥ 7, range of pre-mismatch locomotion speed > 10 cm/s	L2/3: 27 (dMM: 15, hMM: 5, unclassified: 7) L5/6: 10
3A (top boxplot), S3B	Voltage offset recorded from amplifier (this was accidentally omitted in some cases due to human error)	L2/3: 25 (dMM: 13, hMM: 5, unclassified: 8)
4D, 4E, 6H, 6I, S6E, S4H	Open-loop protocol presented during recording	L2/3: 27 (dMM: 14, hMM: 5, unclassified: 8) L5/6: 13
4G	Open-loop protocol presented during recording with 4 different visual flow speeds	L2/3: 15 (dMM: 6, hMM: 4, unclassified: 5)
5D, 6K, 6L, S6F	At least two locomotion onsets in open-loop condition	L2/3: 22 (dMM: 10, hMM: 4, unclassified: 8) L5/6: 13
5E, 5F, 7A, 7B, 7C	At least 25 s of locomotion datapoints in open-loop condition	L2/3: 22 (dMM: 12, hMM: 5, unclassified: 5) L5/6: 12
S3A	At least 10 mismatch trials	L2/3: 22
S4B, S4C, S4D	At least 5 visual stimuli presented during both locomotion and stationary epochs	L2/3: 14 (dMM: 6, hMM: 4, unclassified: 4) L5/6: 11
S4F, S4G	Open-loop protocol presented, and voltage offset recorded from amplifier	L2/3: 25

Table S1. Inclusion criteria and sample sizes for various cell groups and analyses. Related to STAR Methods.
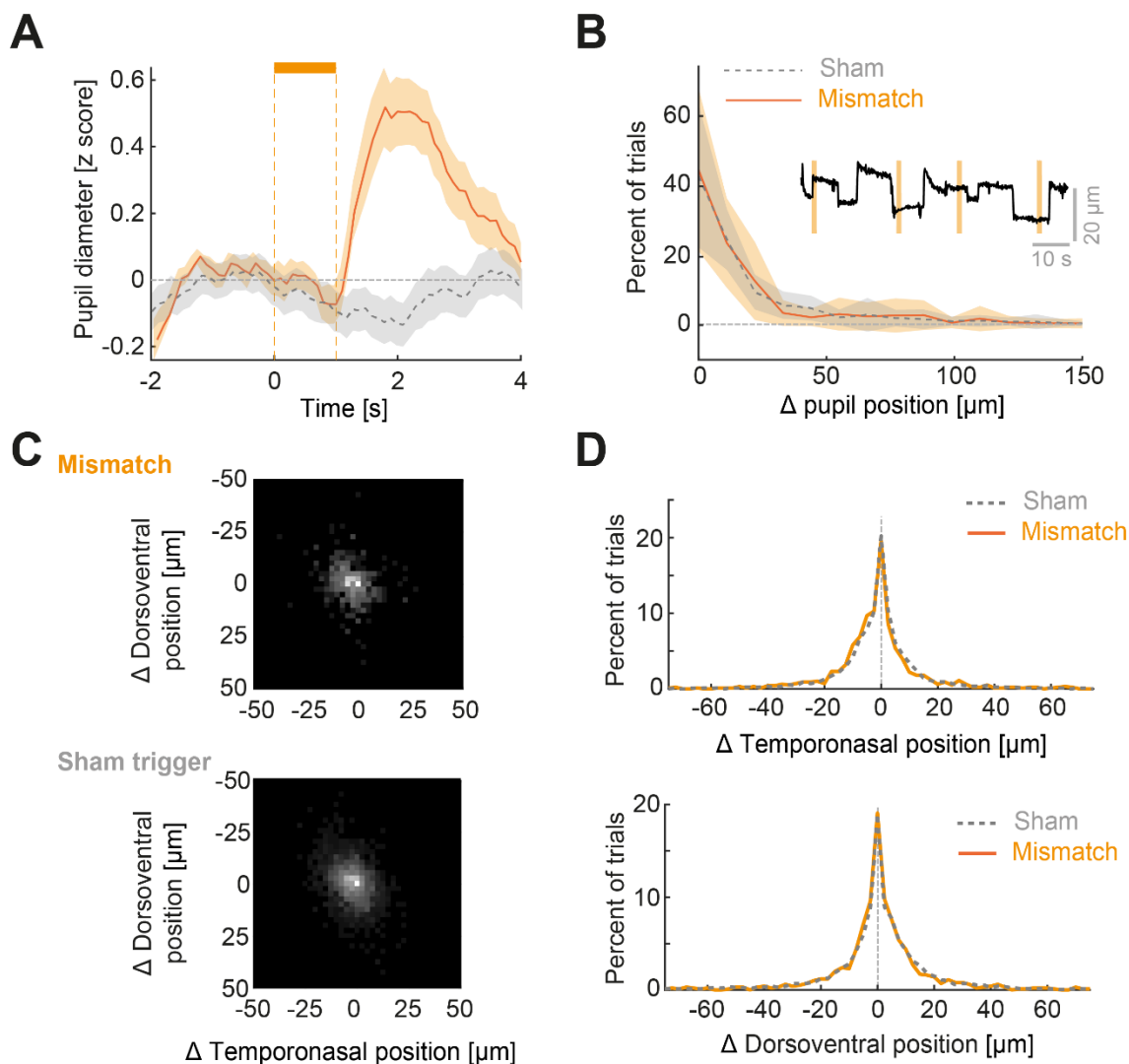
Figure S1. Mismatch evokes pupil dilation but not eye movements. Related to Figure 1.

(**A**) Average pupil diameter changes during mismatch (orange) across 20 mice (separate from mice used for electrophysiology). Gray dashed plot shows the same for sham triggers. Shading shows SEM.

(**B**) Histogram of magnitudes of changes in eye position during mismatch (orange) and sham triggers (gray) averaged across 20 mice. Shading shows SD. Inset shows example pupil x position trace, with orange shading indicating 1 s mismatch stimuli.

(**C**) Two-dimensional histogram of changes in eye position during mismatch trials (top) and sham triggers (bottom) across 1000 trials and 4500 trials, respectively, across 20 mice.

(**D**) Histogram of eye position changes in the temporonasal axis (top) and dorsoventral axis (bottom) across 1000 mismatch trials (orange) and 4500 sham trigger trials (dashed gray) from 20 mice.
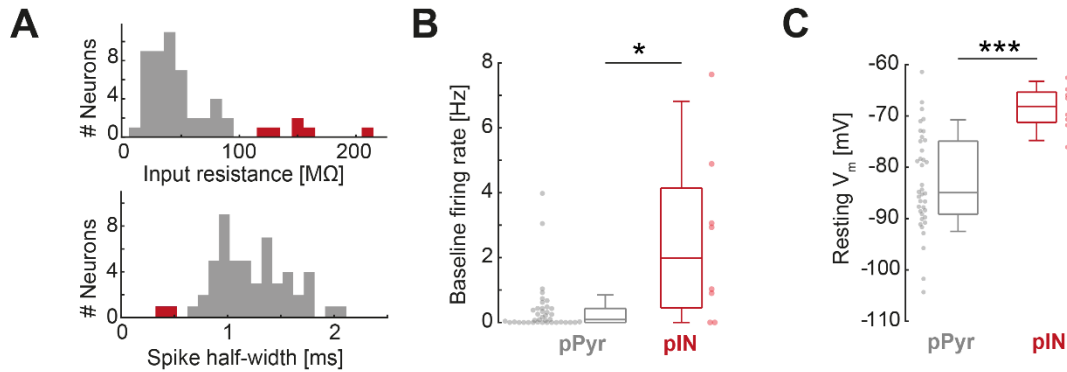
Figure S2. Exclusion of putative interneurons and correlations between mismatch responses and locomotion speed. Related to Figure 1.

(**A**) Distribution of input resistance (top) and spike half-width (bottom) of the entire dataset, regardless of recording depth. Marked in red are neurons we excluded as potential interneurons, either based on input resistance > 100 MΩ or a spike half-width < 0.6 ms. Neurons excluded using each criterion did not overlap. Excluded neurons showed other electrophysiological properties that differed from the remaining dataset and were consistent with interneuron properties (see **B** and **C**).

(**B**) Comparison of baseline firing rate (during stationary periods) for putative excitatory neurons (pPyr) versus the excluded putative interneurons (pIN). Baseline firing rates for putative interneurons were significantly more variable ($p < 10^{-3}$, Brown-Forsythe test), and significantly higher than in putative excitatory neurons ($p < 0.03$, Wilcoxon rank-sum test).

(**C**) As in **B**, but for initial resting membrane potentials recorded just after entry into whole cell mode. Membrane potentials in putative interneurons were significantly less variable ($p < 0.05$, Bartlett test), and significantly more depolarized than for putative excitatory neurons ($p < 10^{-3}$, Wilcoxon rank-sum test).
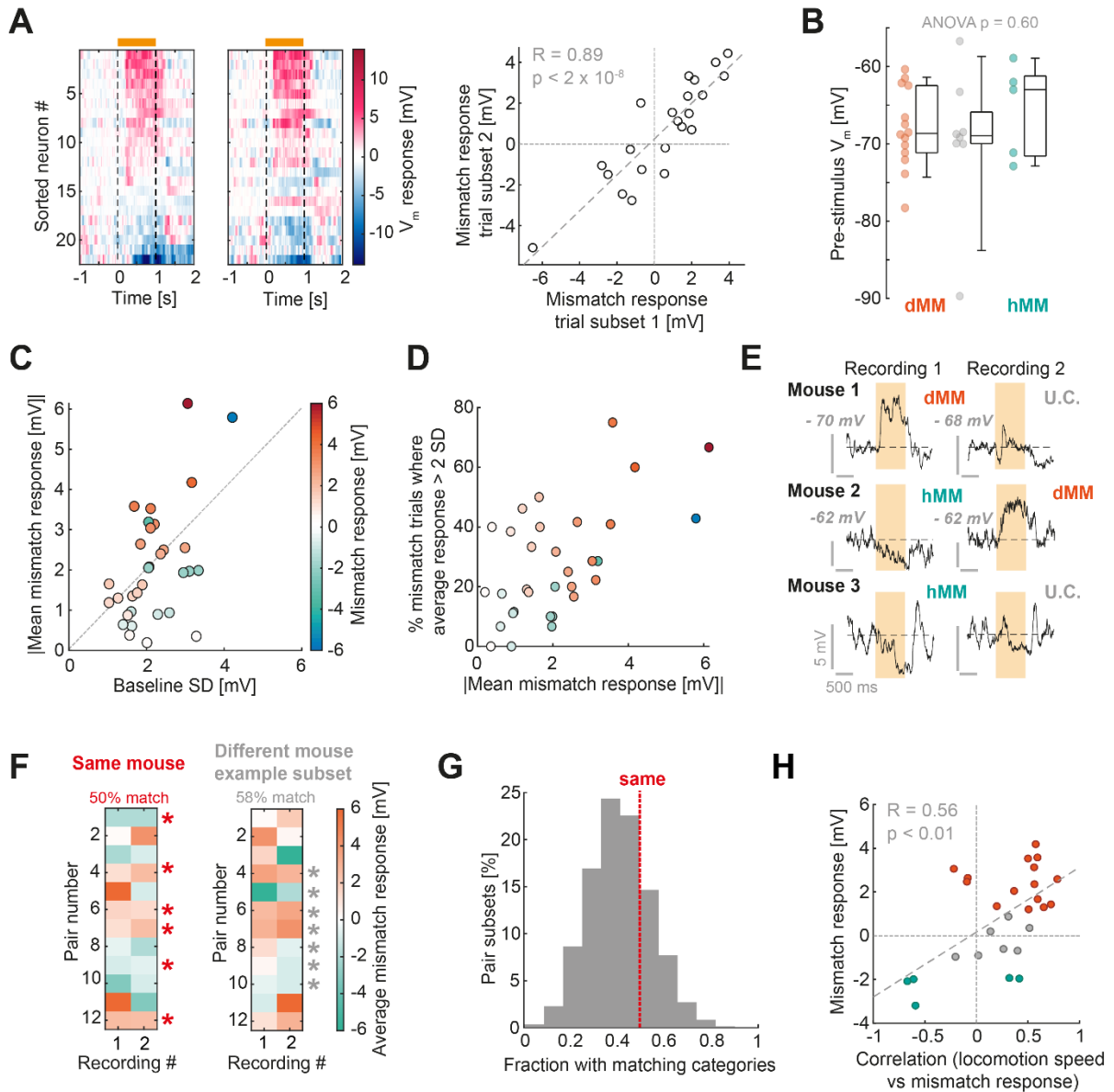
Figure S3. Additional data for mismatch responses in L2/3 neurons. Related to Figure 2.

(**A**) Cross-validation plots for mismatch responses. Left: Heatmaps show average mismatch response for each neuron calculated from non-overlapping subsets of mismatch trials. Neurons are sorted in both heatmaps according to average response to mismatch in the left heatmap. Right: scatter plot between average mismatch response in one trial subset versus average mismatch response in the remaining trials. Only neurons with at least 10 mismatch trials were included in this analysis.

(**B**) Average pre-stimulus membrane potential for the three mismatch response categories (dMM, hMM and unclassified). The pre-stimulus membrane potential is not different for the three groups of neurons and cannot account for the differences in mismatch responses. One-way ANOVA, p = 0.60.

(**C**) Absolute mean mismatch response (averaged over all trials and the entire 1 s window of mismatch presentation) plotted against the standard deviation (SD) of the baseline membrane potential (calculated from sham-triggered 1 s average V_m changes during locomotion – see STAR methods). Points are colored by the mismatch response of the neuron (scale bar as in **G**).

(**D**) Percent of trials showing an average absolute mismatch response exceeding 2 standard deviations of the baseline membrane potential (calculated from sham-triggered 1 s average V_m changes during locomotion – see STAR methods), plotted against absolute mismatch response. Points are colored by the mismatch response of the neuron according to the scale bar in **C.**

(**E**) Example average mismatch responses from pairs of neurons recorded from the same mouse. In grey bold italics indicates mean pre-stimulus voltage (unavailable for mouse 3 as amplifier offset was not recorded). Note: U.C. stands for unclassified.

(**F**) Left: Heatmap showing average mismatch response for all pairs of L2/3 neurons recorded in the same mouse. Asterisks indicate where type of response to mismatch (dMM, hMM, or unclassified) is matching between the two pairs. Left: as for the right heatmap, but for a random subset of pseudopairs recorded in two different mice.

(**G**) Histogram of the fraction of matching mismatch response types for 10,000 random subsets of 12 neurons recorded in different mice. Red dashed line indicates the fraction of matches for neuronal pairs recorded from the same craniotomy. 18% of pseudopairs recorded from different mice had fractions of matches equal to or more than that for the pairs recorded from the same mouse.

(**H**) Scatter plot between the correlation coefficient for the relationship between mismatch response and locomotion speed (as in **Figure 2G**), and average mismatch response. Points are colored according to mismatch response category (dMM: orange, hMM: turquoise, unclassified: gray).
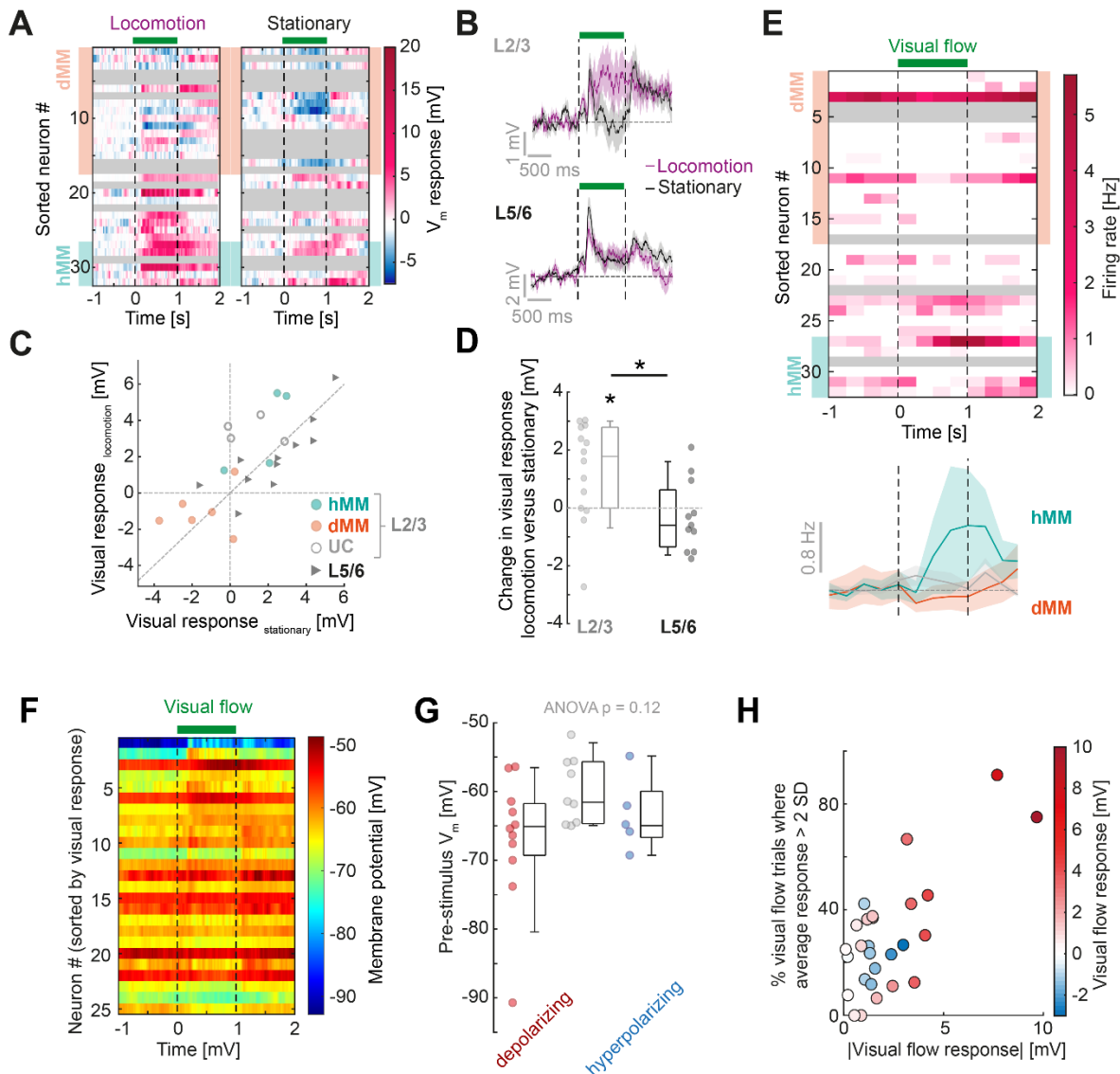
Figure S4. Additional data for visual flow responses in L2/3 neurons. Related to Figure 4.

(**A**) Heatmaps of subthreshold visual flow responses of L2/3 neurons sorted by mismatch response (as in **Figure 2A**), during locomotion (left), or during stationary periods (right). Gray marks neurons for which we did not have at least five visual flow presentations in that condition. Orange shading indicates dMM neurons and turquoise shading indicates hMM neurons.

(**B**) Average visual flow response of 14 L2/3 neurons (top) and 11 L5/6 neurons (bottom) during locomotion (purple) and stationary periods (black). Shading shows SEM. Only neurons with at least five trials in each category were included. Average pre-visual stimulus voltages: L2/3, stationary: -63 ± 5 mV; L2/3, locomoting: -62 ± 8 mV; L5/6, stationary: -65 ± 8 mV; L5.6, locomoting: -60 ± 8 mV;

(**C**) Scatter plot of visual flow responses during stationary periods and visual flow responses during locomotion periods for all neurons with at least five trials in each category. Note: In legend, UC = unclassified.

(**D**) Change in visual flow response between locomotion and stationary periods for L2/3 neurons and L5/6 neurons. L2/3 neurons showed significantly more positive responses during locomotion compared to stationary periods (p < 0.02, paired Student's t-test). L5/6 neurons did not show this effect (p = 0.45, paired Student's t-test). Changes were higher for L2/3 neurons versus L5/6 neurons (p < 0.02, Student's t-test).

(**E**) Top: Heatmap of firing rates of neurons during visual flow stimuli, sorted by mismatch response (as in **Figure 2A**). Gray marks neurons for which we did not have at least five visual flow presentations. Orange shading indicates dMM neurons and turquoise shading indicates hMM neurons. Bottom: Average response across 14 dMM neurons (orange), 5 hMM neurons (turquoise), and the remaining 8 neurons (gray).

(**F**) Heatmap of visual flow responses, sorted according to average visual flow response (most depolarizing at the top), across 27 L2/3 neurons. Responses are not normalized according to baseline membrane potential.

(**G**) Box plot to compare average pre-stimulus membrane potential for neurons which depolarized (> 1 mV visual flow response, red), hyperpolarized (< -1 mV visual flow response, blue), and remaining neurons (gray). One-way ANOVA, p = 0.12.

(**H**) Percent of trials showing an average absolute visual flow response exceeding 2 standard deviations of the baseline membrane potential (calculated from sham-triggered 1 s changes in V_m during both locomotion and stationary periods), plotted against absolute visual flow response. Points are colored by the visual flow response of the neuron.
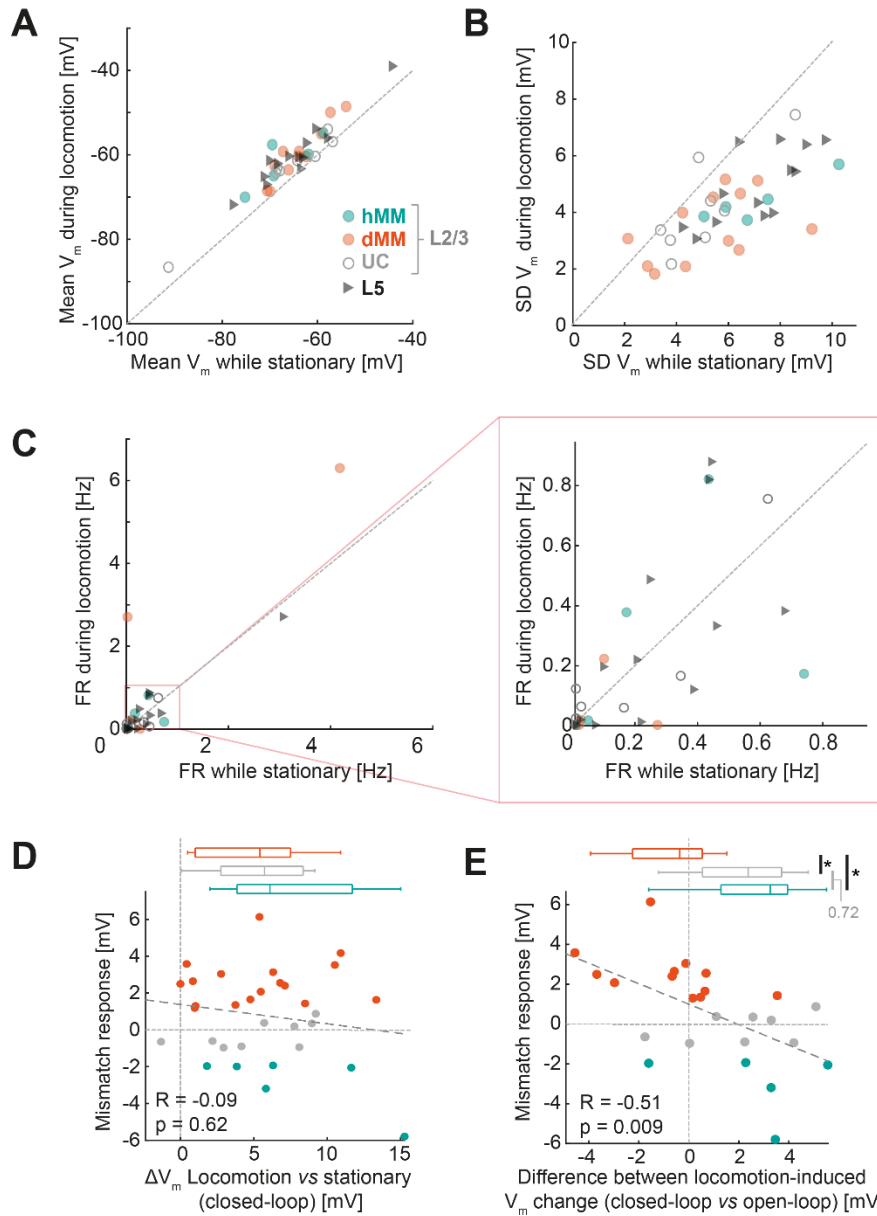
Figure S5. Membrane potential dynamics and firing rate changes between stationary periods and locomotion in open-loop condition. Related to Figure 5.

(**A**) Mean membrane potential (V_m) during stationary periods versus that during locomotion. All neurons showed depolarization of membrane potential during locomotion. Note: In legend, UC = unclassified. Locomotion induces significant depolarization: mean ± SD, ΔV_m = 4.5 ± 2.5 mV, $p < 10^{-10}$, 39 neurons, paired Student's t-test.

(**B**) As in **A**, but for the standard deviation (SD) in membrane potential. Locomotion induces significant reduction in standard deviation: mean ± SD, ΔV_m SD = -1.8 ± 1.5 mV, $p < 10^{-8}$, paired Student's t-test.

(**C**) As in **A**, but for firing rates (FR). Right plot shows an expanded version of the left. Change in firing rates during locomotion: mean ± SD, ΔFR = 0.11 ± 0.62 Hz, $p = 0.28$, paired Student's t-test.

(**D**) Scatter plot of mismatch response versus V_m change during locomotion in closed-loop relative to stationary periods for 32 L2/3 neurons. Points are colored according to mismatch response category (dMM: orange, hMM: turquoise, unclassified: gray). Boxplots above compared data for dMM, hMM and unclassified neurons. One-way ANOVA: $p = 0.51$, F-statistic = 0.7.

(**E**) Scatter plot of mismatch response versus the difference between locomotion-induced V_m change (relative to stationary periods) in closed-loop *vs* open-loop epochs. Positive values indicate more depolarization during locomotion in closed-loop (where visual flow is coupled to locomotion) compared to open-loop. Points are colored according to mismatch response category (dMM: orange, hMM: turquoise, unclassified: gray). Boxplots above compared data for dMM, hMM and unclassified neurons. One-way ANOVA: $p < 0.02$; F-statistic = 5.4; dMM vs hMM: $p < 0.02$, dMM vs unclassified: $p < 0.02$, hMM vs unclassified: $p = 0.72$, Student's t-test.
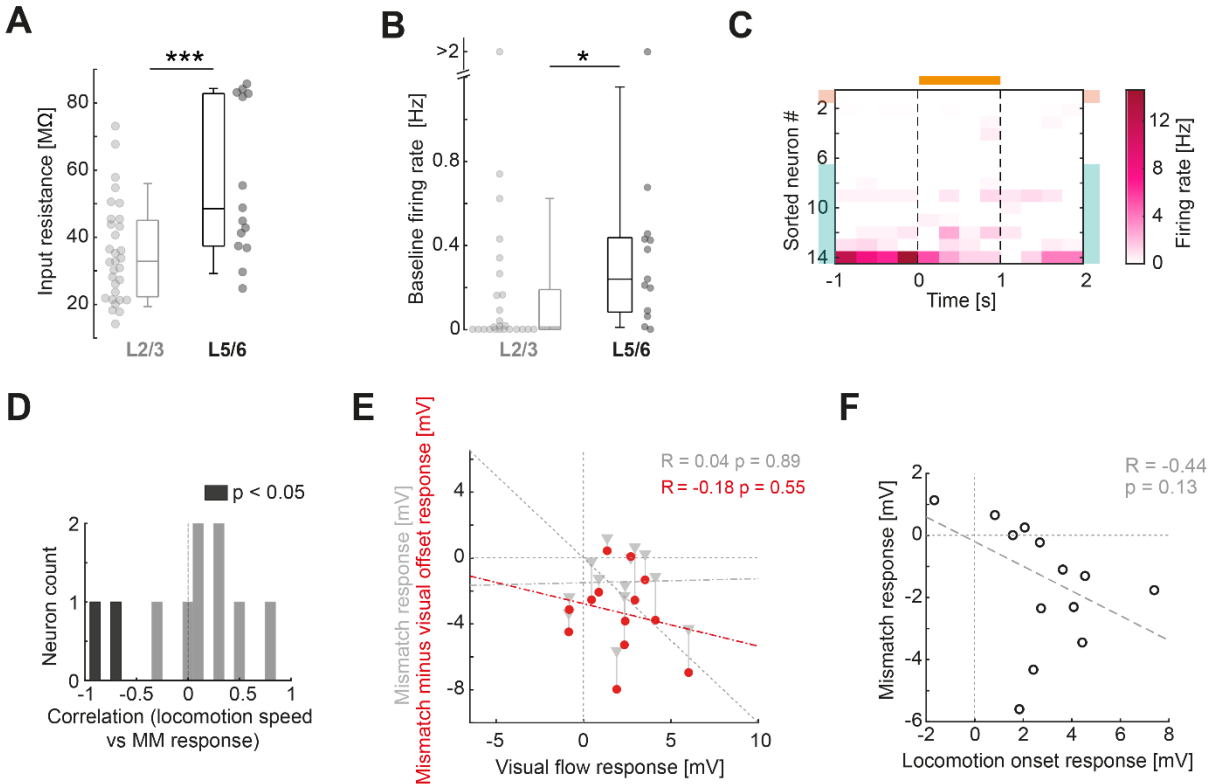
Figure S6. Comparison of properties between putative L5/6 and L2/3 excitatory neurons. Related to Figure 6.

(**A**) Input resistance was significantly higher in L5/6 neurons than in L2/3 neurons ($p < 0.001$, Student's t-test).

(**B**) Baseline firing rate was significantly higher in L5/6 neurons than in L2/3 neurons ($p < 0.02$, Wilcoxon rank-sum test).

(**C**) Heatmap of average spike counts aligned to mismatch events for L5/6 neurons. Heatmap is sorted according to subthreshold mismatch responses, as in **Figure 6.** Orange shading indicates dMM neurons, and turquoise shading indicates hMM neurons.

(**D**) Histogram of Pearson's correlation coefficients between locomotion speed and mismatch response for L5/6 neurons. In dark gray are counts of neurons with a significant correlation ($p < 0.05$).

(**E**) Scatter plot between average visual response and average mismatch response (gray triangles) for 13 L5/6 neurons. For red data points, visual flow offset responses were subtracted from the mismatch responses. Dashed gray and red lines are linear fits to the respective data.

(**F**) Scatter plot between average locomotion onset response (0 s to 6 s after locomotion onset) and mismatch response for 13 L5/6 neurons
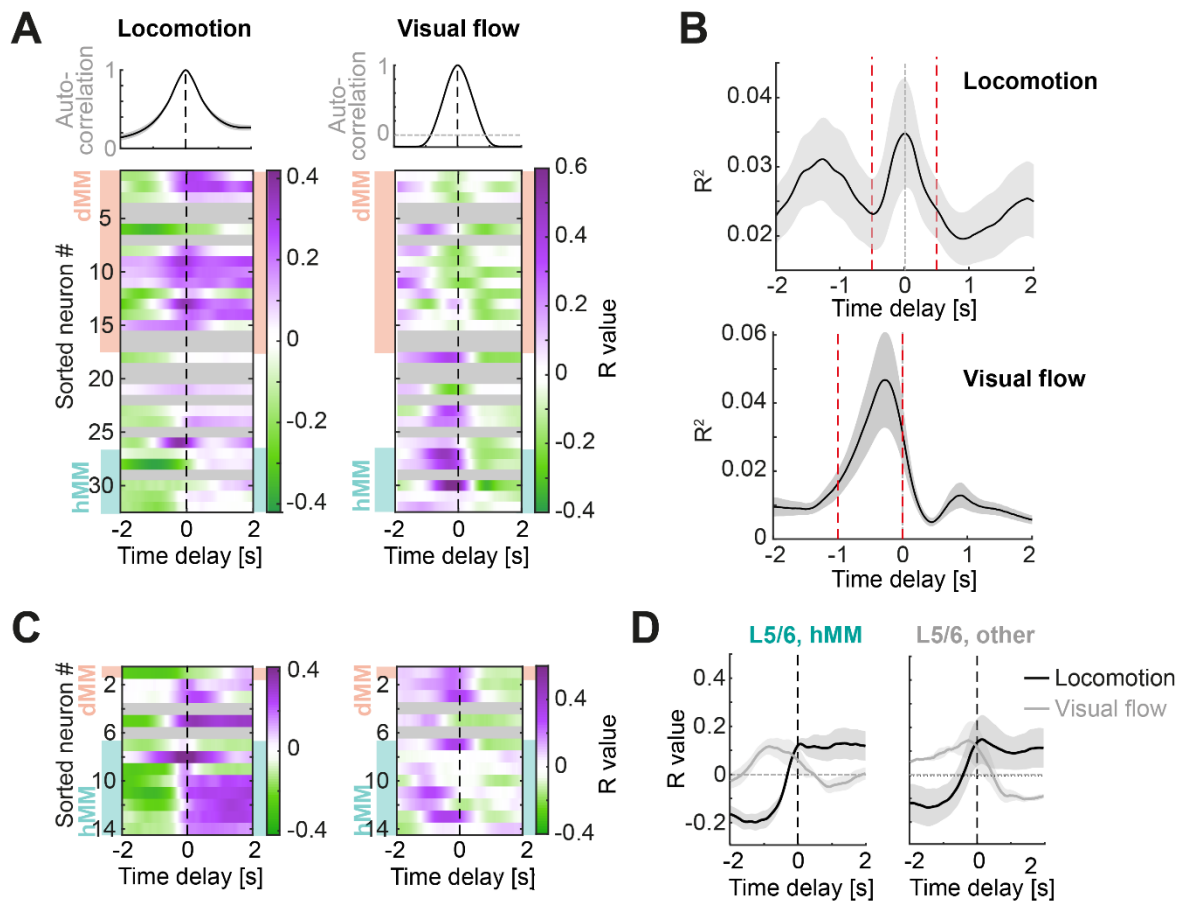
Figure S7. Additional data for cross-correlations between visual flow, locomotion and membrane potential. Related to Figure 7.

(**A**) Top: Average autocorrelation for locomotion speed (left) and visual flow speed (right). Shading indicates SEM over neurons. Bottom: Heatmaps show cross-correlations for each L2/3 neuron between locomotion speed sand membrane potential (left), and visual flow speed and membrane potential (right). Heatmaps are sorted by mismatch response as in **Figure 2A**. All analyses excluded stationary periods. Note: for all panels, negative time values indicate a lag of V_m relative to locomotion/visual flow speed.

(**B**) Average R^2 for all cross-correlations (L5/6 and L2/3 pooled, n = 34) for locomotion speed and V_m (top), and visual flow speed and V_m (bottom). Red dashed lines indicate 1 s window used to calculate the average correlation for each neuron (as plotted in **Figure 7**) – approximately centered around the peak R^2 for locomotion and visual flow separately.

(**C**) As in **A**, but for L5/6 neurons.

(**D**) Average cross-correlations between locomotion speed (black) or visual flow speed (gray) and membrane potential for the 7 L5/6 neurons which hyperpolarize during mismatch (hMM), and the remaining 5 neurons ('other', including 2 depolarizing neurons).