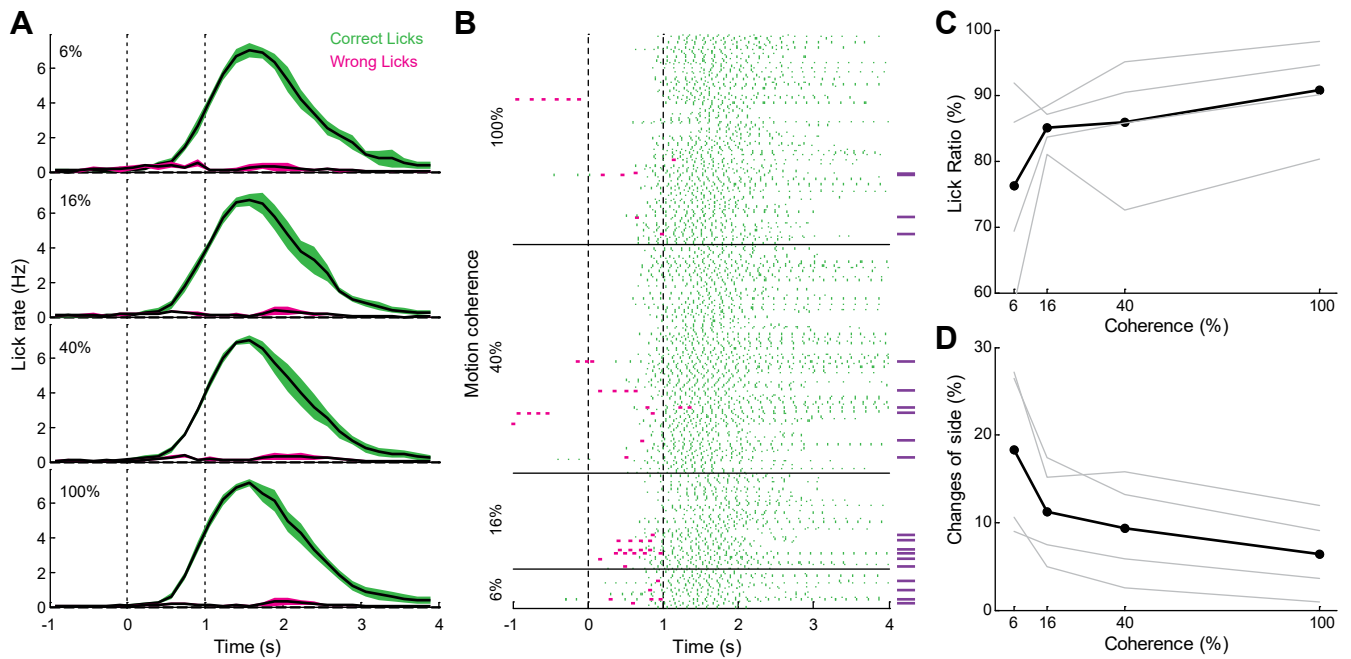


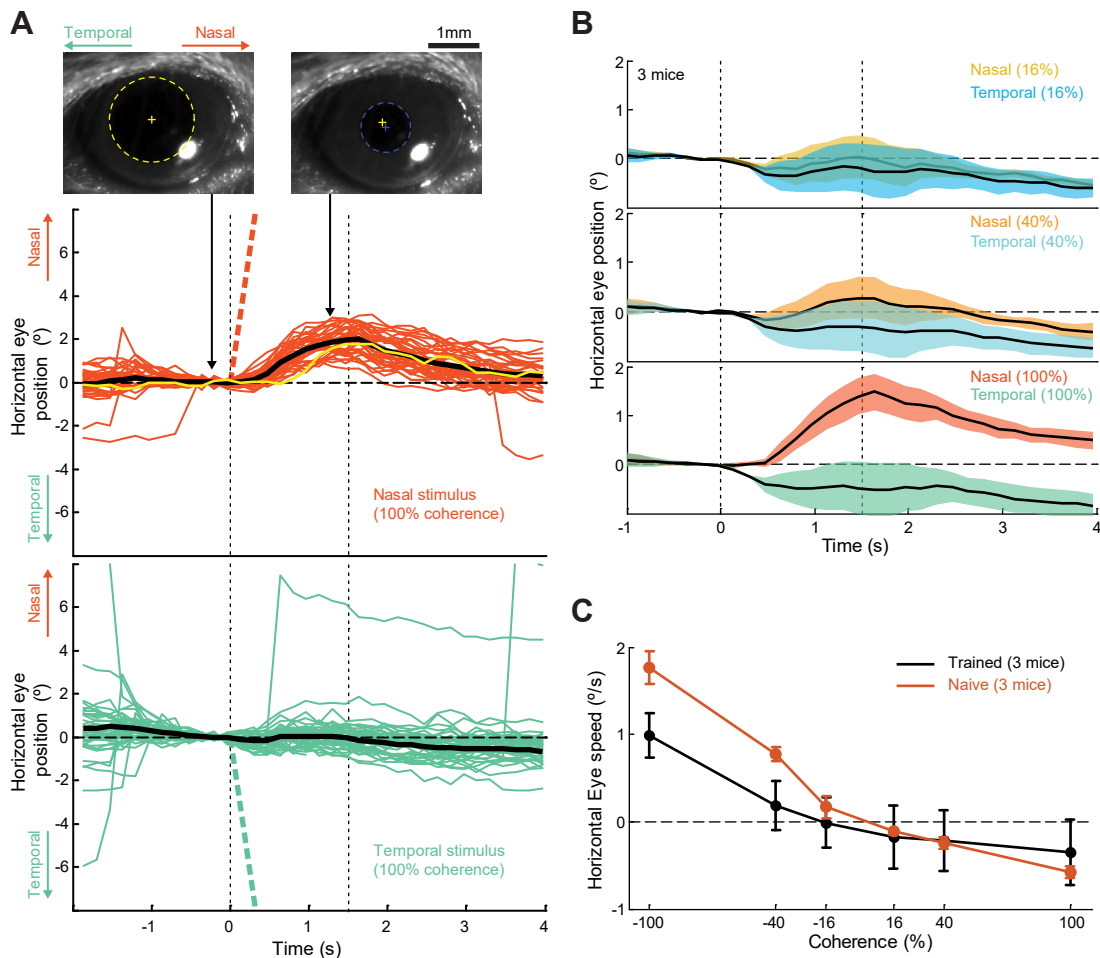
**Figure S1. Mice show no differences in perceptual outcomes between nasal and temporal directions, related to Figure 1.**

**(A)** Accuracy for the 8 different stimuli (nasal and temporal directions and 4 levels of motion coherence). Black thick line and circles, population mean; gray lines, individual animals; orange line, animal with example session in Figure 1D-E. **(B)** Same as in **(A)** but for reaction time defined as time from stimulus onset until the first choice lick (STAR Methods).

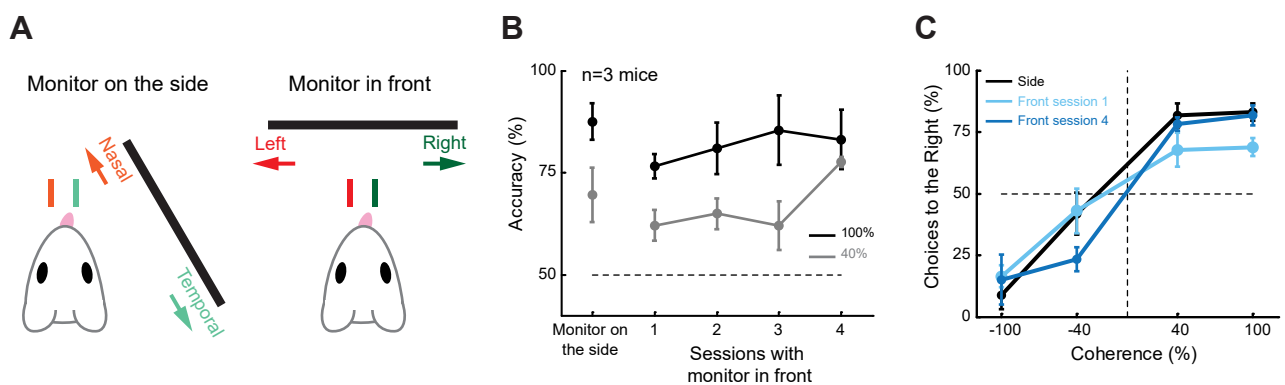


**Figure S2. Mice alternate licking sides more often for harder stimuli, related to Figure 1.**

**(A)** Average licking traces for correct (light green) and wrong (pink) licks sorted by coherence. Only correct trials are shown (4 mice, same as in Figures 1F and 2A). Black line, mean; shaded area, SEM. Black dashed lines, stimulus onset and offset. **(B)** Correct (light green) and wrong (pink) licks in correct trials for example session in Figure 1D. Purple lines, trials in which the mouse alternated licking during stimulus presentation. **(C)** Lick ratio (STAR Methods) as a function of motion coherence for sessions in Figures 1F and 2A. **(D)** Percentage of trials in which mice alternated licking during the stimulus presentation as a function of motion coherence ( $p = 0.023$ , repeated measurements ANOVA). Circles and black line, mean; gray lines, individual animals.

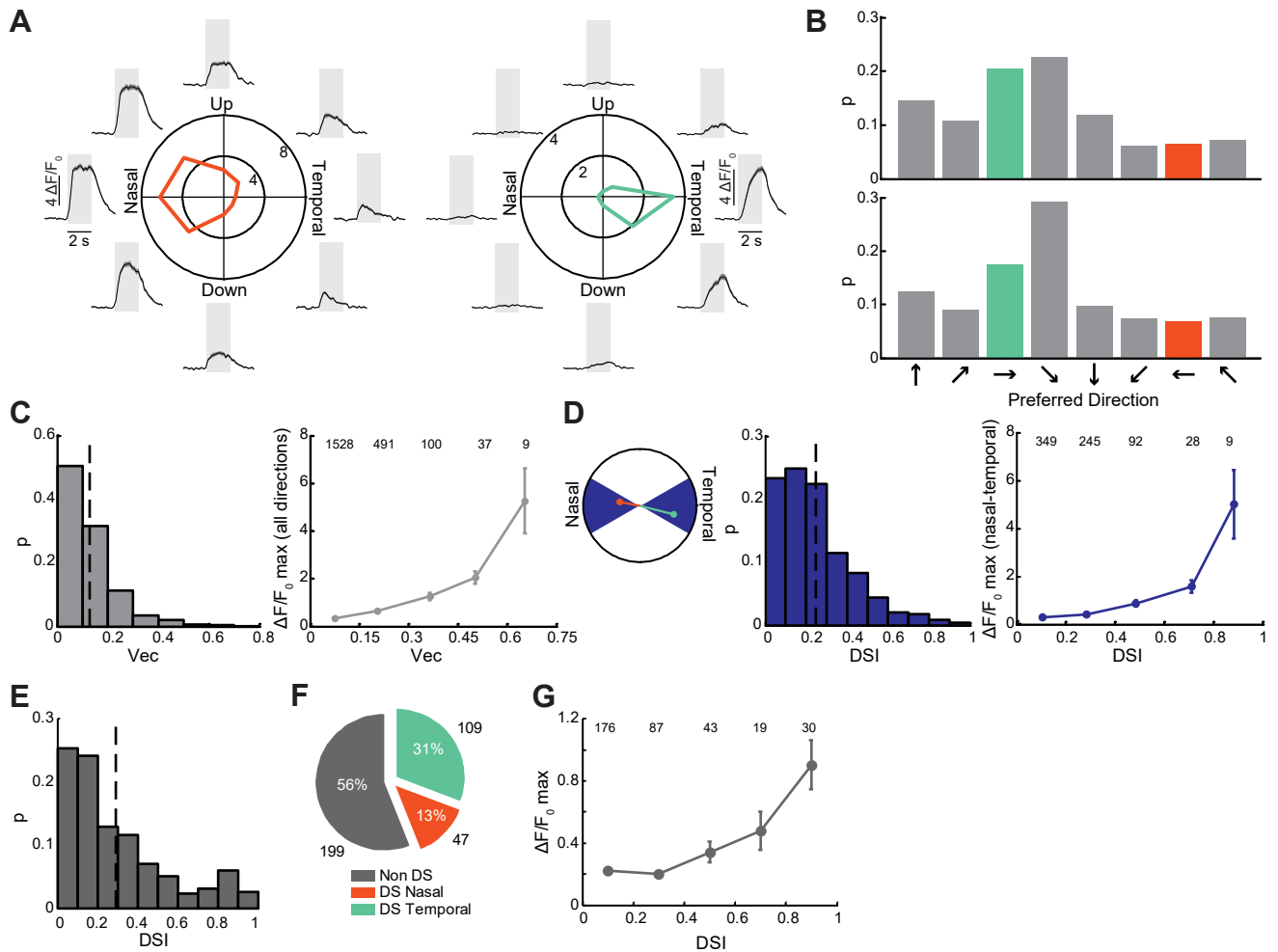


**Figure S3. Eyes are mostly stable upon presentation of the stimulus, related to Figure 2.** **(A)** Top, frames of eye movie during a trial at 200ms before stimulus onset (left, yellow) and 1.3s after stimulus onset (right, blue). Middle, traces of eye horizontal position for all the 100% coherence nasal trials in one session. Bottom, same but for the 100% coherence temporal trials. Colored lines, individual trials; thick black line, session average.; yellow line, example trial from eye movie frames shown above. Thick colored dashed lines, slope corresponding to the speed of the stimulus (25°/s). Black dashed lines, stimulus onset and offset. **(B)** Population traces of eye horizontal position (3 mice). Trials are sorted by coherence and side. Black line, mean; shade area, SEM. **(C)** Mean horizontal eye speed during stimulus presentation per stimulus type. Black, trained animals during motion discrimination task (from **(B)**); orange, naive animals passively viewing random dot stimuli (3 mice). Circles, mean; errorbars, SEM.



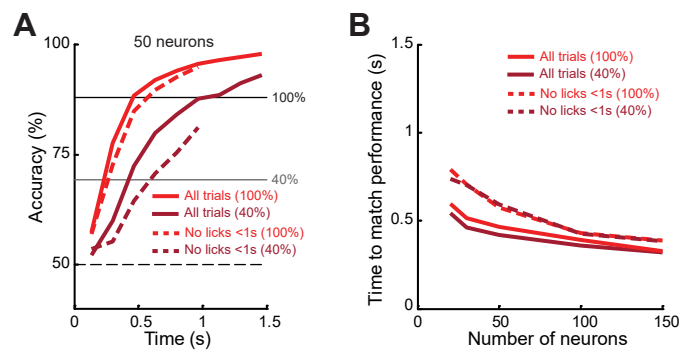
**Figure S4. Motion discrimination task is generalizable to stimuli in the binocular visual field, related to Figure 2.**

(A) Left, scheme with normal position of the monitor. Mice choose the left lickport to report RDK moving in the nasal direction and the right lickport for RDK moving in the temporal direction. Right, scheme with the monitor in the binocular visual field. Mice choose the left lickport to report RDK moving left and the right lickport for RDK moving right. (B) Performance of the animals in the last session with the monitor on the side and in the sessions with the monitor in front for both the 100% (black) and 40% (gray) coherence stimuli. (C) Population psychometric curve for the monitor on the side and for the first and fourth sessions with the monitor in front. Circles, mean; errorbars, SEM.



**Figure S5. RDK stimuli strongly drive highly DS L2/3 neurons, related to Figure 3 and 5.**

**(A)** Left, example tuning curve of a L2/3 V1 DS neuron tuned to the nasal direction. Right, same but for a DS neuron tuned to the temporal direction. Mean calcium traces surround each polar plot, corresponding to a stimulus presentation in that direction. Gray area, stimulus presentation. Polar plot, mean  $\Delta F/F_0$  during the stimulus period. **(B)** Distribution of preferred directions for DS neurons. Top, preferred direction defined as the one eliciting the largest response ( $p = 5.48 \times 10^{-35}$ , Chi square gof test for uniform distribution). Bottom, preferred direction defined by the vector sum of all the responses binned in  $45^\circ$  bins ( $p = 1.36 \times 10^{-52}$ , Chi square gof test for uniform distribution). Green and orange, temporal and nasal preferring DS neurons. **(C)** Left, distribution of directional tuning measured as normalized vector sum length (Vec) for visually responsive L2/3 V1 neurons. Dashed line, mean of the distribution (0.13). Right, Population mean  $\Delta F/F_0$  max as a function of Vec. Neurons were binned in Vec intervals of 0.15 ( $p = 6.62 \times 10^{-162}$ , ANOVA). Circle, mean; errorbars, SEM. Numbers on top correspond to the number of neurons in each bin. **(D)** Left, scheme showing the normalized vector sum of the example neurons in A. Blue areas, range of angles of vector sum of responses of neurons considered as preferring horizontal motion. Middle, distribution of DSI (calculated considering only the nasal and the temporal stimuli) for visually responsive neurons preferring horizontal motion. Dashed line, mean of the distribution (0.25). Right, Population mean  $\Delta F/F_0$  max (considering only the nasal and temporal stimuli) as a function of DSI for neurons preferring horizontal motion. Neurons were binned in DSI intervals of 0.2 ( $p = 1.44 \times 10^{-66}$ , ANOVA). Circle, mean; errorbars, SEM. Numbers on top correspond to the number of neurons in each bin. These plots are equivalent to those in Figure 5D,E but restricted to neurons that are tuned to horizontally-moving motion when tested with 8 directions. **(E)** Distribution of DSI for visually responsive neurons during behavior. Dashed line, mean of the distribution (0.29). **(F)** Relative abundance of non-DS, nasal or temporal preferring DS neurons during behavior. **(G)** Population mean  $\Delta F/F_0$  max as a function of DSI during behavior. Neurons were binned in DSI intervals of 0.2 ( $p = 7.1 \times 10^{-18}$ , ANOVA). Circle, mean; errorbars, SEM. Numbers on top correspond to the number of neurons in each bin.



**Figure S6. Ideal observer analysis in trials with late licking, related to Figure 6.**

**(A)** Performance of a population decoder trained using a SVM during the stimulus presentation with 50 neurons. Unbroken colored lines, same data as Figure 6B. Dashed colored lines, trials in which animals licked during the first 1s of stimulus presentation were removed for training and testing the decoder. Horizontal lines, mean animal performance of the imaging sessions. **(B)** Time for the population decoder to match animal performance using population sizes of 20, 30, 50, 100 and 150 neurons. Unbroken colored lines, data from Figure 6C. Dashed colored lines, after removing trials with licks during the first 1s after stimulus presentation.