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Supplemental information

The locus coeruleus mediates behavioral flexibility

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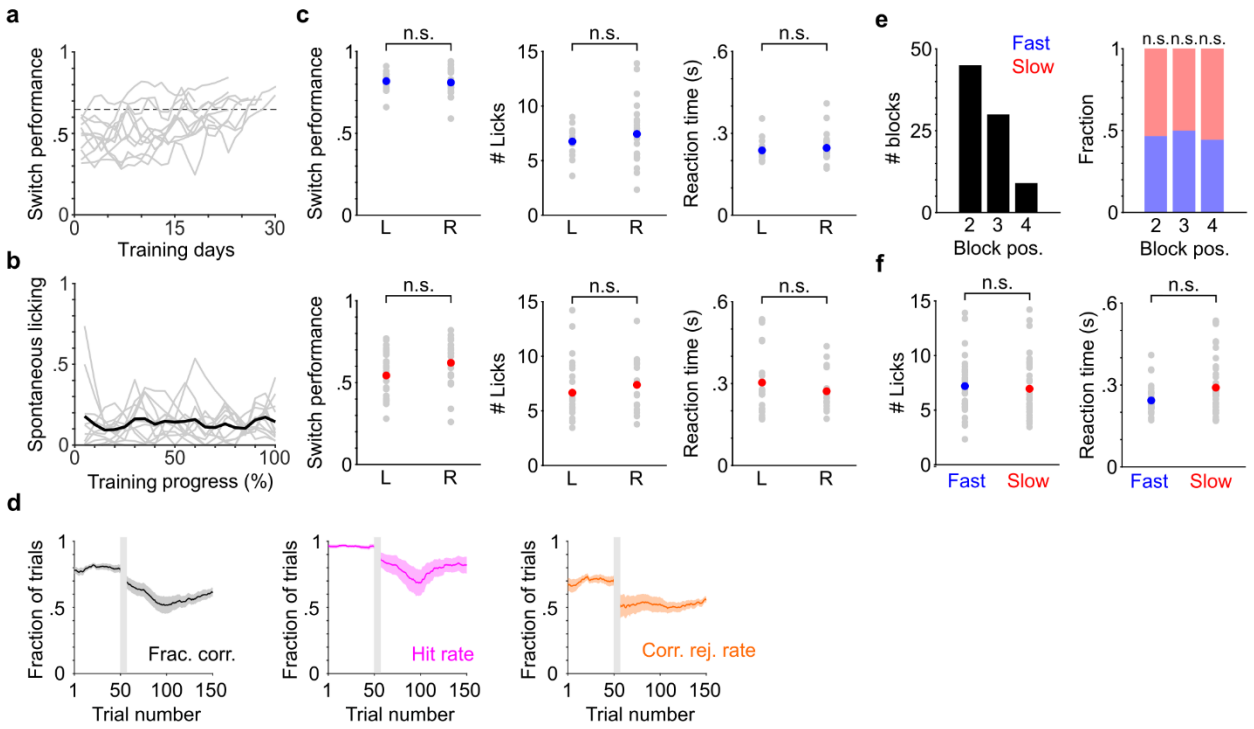

Figure S1. Quantifying mouse behavior in the novel tactile-based rule-shift detection task

(a) Switch performance across training days (n = 11 mice). Normalized training progressions from the same data are shown in Fig. 1d.

(b) False alarm rate (spontaneous licking rate) on catch trials during training. On catch trials no whisker stimulation was delivered.

(c) Behavioral variables quantified for Left Go blocks (L) and Right Go blocks (R) in fast (Blue, Top: L, n = 14; R, n = 26) and slow (Red, Bottom: L, n = 26; R, n = 18) switches. Fast switches: switch performance, L vs. R: 0.82 ± 0.02 vs. 0.81± 0.01, p = 0.71, rank sum = 301; Number of licks, L vs. R: 6.76 ± 0.38 vs. 7.44 ± 0.53, p = 0.42, rank sum = 258; Reaction time, L vs. R: 0.24 ± 0.01 vs. 0.25 ± 0.01 s, p = 0.41, rank sum = 257. Slow switches: switch performance, L vs. R: 0.54 ± 0.03 vs. 0.62 ± 0.04, p = 0.095, rank sum = 515; Number of licks, L vs. R: 6.66 ± 0.53 vs. 7.38 ± 0.58, p = 0.25, rank sum = 536; Reaction time, L vs. R: 0.30 ± 0.02 vs. 0.27 ± 0.02 s, p = 0.59, rank sum = 608, two-tailed Wilcoxon rank-sum test. Gray dots represent individual blocks, red and blue dots represent mean. Number of licks and reaction time were quantified in hit trials. Reaction time was calculated as the latency from whisker stimulation onset to the time of the first lick.

(d) Group average behavior (n = 11 mice, mean ± s.e.m.) around rule switch for overall performance, hit rate and correct rejection rate (Left to Right). Vertical gray bars indicate cueing trials. 50 trials before rule switch and 100 trials after rule switch were included. Correct rejection rate appeared to recover more slowly than hit rate.

(e) The distribution of block positions within a session for the blocks shown in Fig. 1f-h (Left, n = 84), and the proportion of fast (blue) and slow (red) blocks in each position within a session (Right). Block 2: Fast vs. Slow: 0.47 vs. 0.53, p = 0.53, t-stat = 0.40; block 3: Fast vs. Slow: 0.50 vs. 0.50, p = 1, t-stat = 0; block 4: Fast vs. Slow: 0.44 vs. 0.56, p = 0.64, t-stat = 0.22, chi-squared test.

(f) Comparison of lick responses between fast and slow switch blocks (40 vs. 44 blocks). Number of licks, Fast vs. Slow: 7.20 ± 0.37 vs. 6.96 ± 0.39, p = 0.38, rank sum = 1800; Reaction time, Fast vs. Slow: 0.24 ± 0.01 vs. 0.29 ± 0.02 s, p = 0.056, rank sum = 1486, two-tailed Wilcoxon rank-sum test. Gray dots represent individual blocks, blue and red dots represent mean.

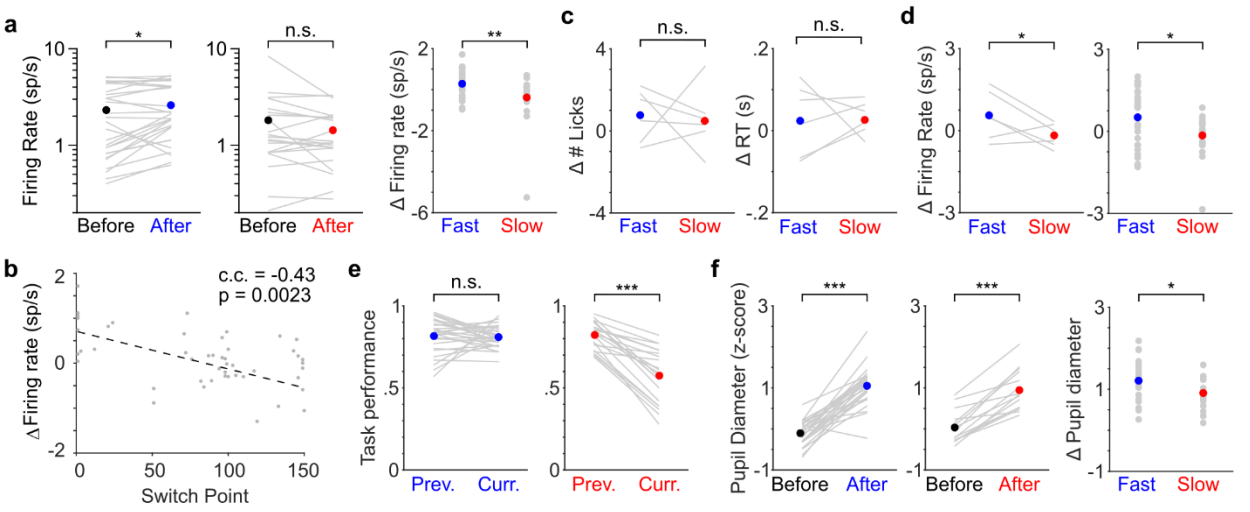

Figure S2. Changes in baseline LC activity reflect the ability to switch and are not a direct effect of licking

(a) Baseline LC activity during the Before and After periods for fast (Left: Before vs. After: 2.31 ± 0.31 vs. 2.60 ± 0.30 spikes/s, p = 0.03, signed rank = 109.5, n = 28) and slow (Middle: Before vs. After: 1.81 ± 0.38 vs. 1.43 ± 0.20 spikes/s, p = 0.10, signed rank = 163, n = 21, two-tailed Wilcoxon signed-rank test) switches. Changes in baseline activity (ΔFiring rate: After - Before) were higher during fast switches than slow switches (Right: Fast vs. Slow: 0.29 ± 0.12 vs. -0.38 ± 0.26 spikes/s, p = 0.0074, rank sum = 833, two-tailed Wilcoxon rank-sum test).

(b) The relationship between behavioral switch point and the changes in baseline LC activity as in Fig. 2i but using a parameter set (1-s baseline window, 70-trial moving window and 90% performance threshold) that maximized the spread of switch point to better demonstrate the variability in switching behavior. Data spread was calculated by comparing the distribution of switch point defined by each set of trial window and performance threshold listed in Table S1 to a reference even distribution. The parameter set that produced the smallest difference (most similar to the even distribution) was chosen.

(c) Changes in number of licks (Left: Δ# licks, After – Before, Fast vs. Slow: 0.79 ± 0.52 vs. 0.56 ± 0.62, p = 0.96, t-stat = -0.05, n = 6) and reaction time (Right: ΔRT, Fast vs. Slow: 0.03 ± 0.04 vs. 0.03 ± 0.02 s, p = 0.83, t-stat = 0.22, n = 6, two-tailed t-test) for the paired fast-slow blocks shown in Fig. 2h.

(d) Changes in baseline LC activity for the paired fast-slow blocks (n = 6) as in Fig. 2h (Left, Fast vs. Slow: 0.56 ± 0.36 vs. -0.18 ± 0.18 spikes/s, p = 0.041, t-stat = 2.16, two-tailed t-test) and all blocks as in Fig. S2a (Right, Fast vs. Slow: 0.51 ± 0.20 vs. -0.15 ± 0.17 spikes/s, p = 0.037, rank sum = 722, 26 fast and 21 slow switch blocks, two-tailed Wilcoxon rank-sum test) quantified in hit trials only. For c and d, there were no hit trials during the Before period of 2 blocks from the original full dataset (49 blocks), and they were removed from this analysis. 1 block was included in the original paired analysis (7 fast-slow pairs), so the associated pair was removed from this analysis.

(e) Left: Comparison of task performance quantified in blocks immediately preceding the fast blocks (Previous) and quantified in the fast blocks (Current). Previous vs. Current: 0.82 ± 0.02 vs. 0.81 ± 0.01, p = 0.29, signed rank = 157, n = 28. Right: Comparison of task performance quantified in blocks immediately preceding the slow blocks (Previous) and quantified in the slow blocks (Current). Previous vs. Current: 0.82 ± 0.02 vs. 0.57 ± 0.03, p = 5.9e-5, signed rank = 0, n = 21. Two-tailed Wilcoxon signed-rank test.

(f) Pupil diameter during the Before and After periods for fast (Left, Before vs. After: -0.10 ± 0.06 vs. 1.05 ± 0.09 s.d., p = 4.7e-6, signed rank = 404, n = 28) and slow (Middle, Before vs. After: 0.045 ± 0.08 vs. 0.93 ± 0.13 s.d., p = 1.8e-4, signed rank = 188, n = 19, two-tailed Wilcoxon

signed-rank test) switches. Changes in pupil diameter (ΔPupil diameter: After - Before) was higher during fast switches than slow switches (Right, Fast vs. Slow: 1.15 ± 0.10 vs. 0.88 ± 0.11 s.d., $p = 0.04$, rank sum = 751, two-tailed Wilcoxon rank-sum test). 2 slow blocks with poor pupil tracking were excluded from this analysis. Pupil diameter was calculated as the average pupil diameter during a baseline pupil window. The baseline pupil window was defined as a 1-s window starting 1.5 s after the start of the baseline LC window, based on the time lag between LC activity and pupil response (Yang et al., 2021).

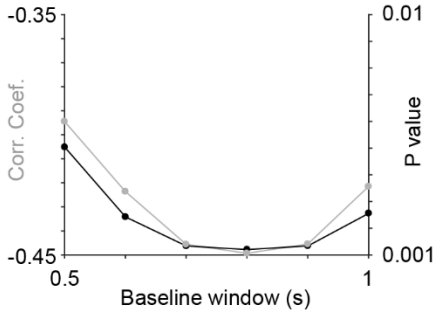

Figure S3. Testing the robustness of the relationship between behavioral switch point and LC activity by varying the baseline window size

Correlation coefficient (gray) and the associated P value (black) as in Fig. 2i, j by varying baseline window size from 0.5 s to 1 s to quantify baseline LC activity (always ending at the onset of whisker stimulation). We used a 50-trial moving window and 85% performance threshold in this analysis.

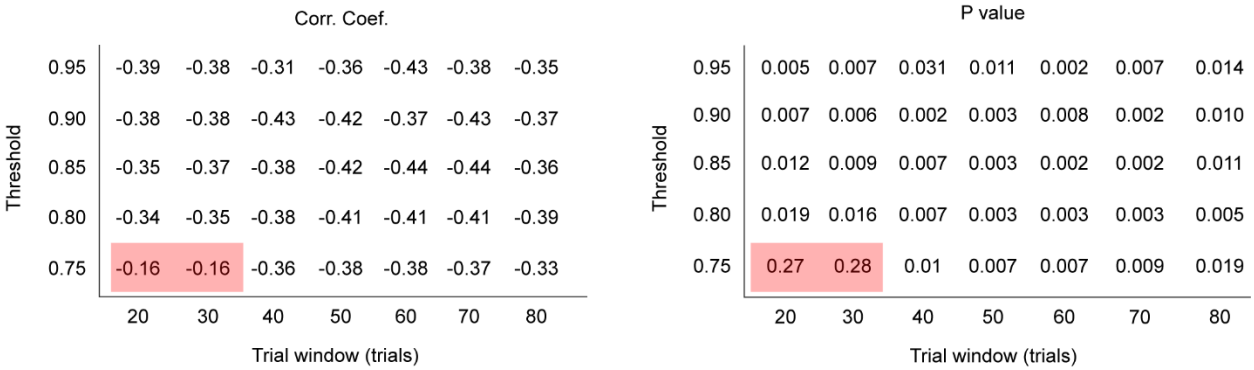

Table S1. Testing the robustness of the relationship between behavioral switch point and LC activity by varying trial window size and performance threshold

Correlation coefficient and the associated P value as in Fig. 2i, j by varying trial window size and performance threshold to determine behavioral switch point (1-s baseline window). The relationship between LC activity and task switching held in the great majority of cases. It became not significant only when both trial window and performance threshold were below critical values (lower left corner).

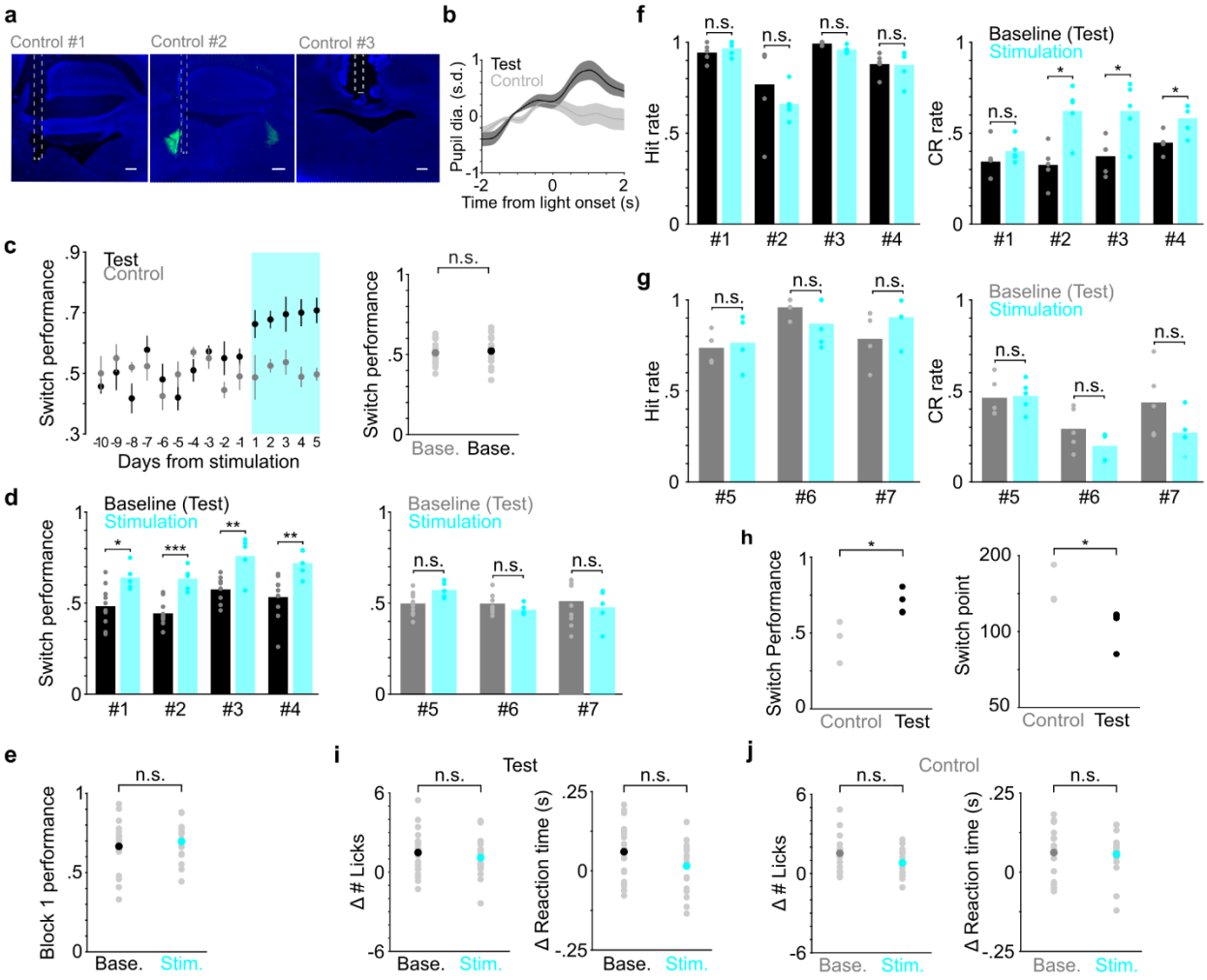

Figure S4. Activating LC facilitates flexible task switching

(a) Histological sections of the control group mice (n = 3) showing optical fiber off-targeted the LC (green, when present). Scalebars represent 200 µm. Fiber implant in control #1 was ~300 µm posterior to the LC. Fiber implant in control #2 was medial and ventral to the LC. Fiber implant in control #3 was ~400 µm posterior to the LC, and apparently medial and dorsal. As a result, LC was not stimulated by blue light in the control group. Control #1-3 correspond to mouse #5-7 in the following panels and Fig. 3e.

(b) Pupil response to optical stimulation from the remaining 1 test mouse and 1 control mouse quantified during miss trials (not confounded by licking).

(c) Left: Group average switch performance for the test and control groups during an extended baseline period (10 consecutive days prior to stimulation) and optical stimulation period (5 consecutive days, cyan). Day -1 represents the last day without stimulation. Day 1 represents the first day with stimulation. Right: Switch performance during the 10-day baseline period for the test (n = 40 blocks) and control (n = 30 blocks) groups (Test vs. Control: 0.52 ± 0.02 vs. 0.51 ± 0.02, p = 0.62, rank sum = 255, two-tailed Wilcoxon rank-sum test).

(d) Switch performance for individual mice (as in Fig. 3d, e), but compared between a 10-day baseline period and 5-day stimulation period (cyan) for the test (Left, n = 4; Baseline vs. Stimulation, Mouse #1: 0.48 vs. 0.64, p = 0.013; Mouse #2: 0.44 vs. 0.63, p = 0.001; Mouse #3: 0.58 vs. 0.76, p = 0.008; Mouse #4: 0.53 vs. 0.72, p = 0.0060, Permutation test) and control (Right,

n = 3; Mouse #5: 0.50 vs. 0.58, p = 0.060; Mouse #6: 0.50 vs. 0.46, p = 0.31; Mouse #7: 0.52 vs. 0.48, p = 0.57, Permutation test) groups.
(e) Comparison of task performance in block 1 between Baseline (n = 20) and Stimulation (n = 20) sessions for the test group (Baseline vs. Stimulation: 0.67 ± 0.03 vs. 0.70 ± 0.02, p = 0.54, rank sum = 387, two-tailed Wilcoxon rank-sum test). 5 sessions per condition per mouse.
(f) Hit rate (Left) and correct rejection rate (Right) for individual mice in the test group (n = 4). Hit rate, Baseline vs. Stimulation, Mouse #1: 0.94 vs. 0.97, p = 0.45; Mouse #2: 0.77 vs. 0.66, p = 0.44; Mouse #3: 0.99 vs. 0.96, p = 0.49; Mouse #4: 0.88 vs. 0.87, p = 0.90, Permutation test; Correct rejection rate, Baseline vs. Stimulation, Mouse #1: 0.34 vs. 0.40, p = 0.32; Mouse #2: 0.32 vs. 0.62, p = 0.023; Mouse #3: 0.37 vs. 0.62, p = 0.036; Mouse #4: 0.45 vs. 0.58, p = 0.024, Permutation test.
(g) Same as in (e) but for the control group (n = 3). Hit rate, Baseline vs. Stimulation, Mouse #5: 0.74 vs. 0.77, p = 0.71; Mouse #6: 0.96 vs. 0.87, p = 0.18; Mouse #7: 0.79 vs. 0.91, p = 0.21, Permutation test; Correct rejection rate, Baseline vs. Stimulation, Mouse #5: 0.47 vs. 0.48, p = 0.88; Mouse #6: 0.29 vs. 0.20, p = 0.13; Mouse #7: 0.44 vs. 0.27, p = 0.10, Permutation test.
(h) Comparison of switch performance (Left) and switch point (Right) across mice between the control group (n = 3) and the test group (n = 4) during optical stimulation. Switch performance, Control vs. Test, p = 0.02, t-stat = 3.0; Switch point: Control vs. Test, p = 0.05, t-stat = -2.5, two-tailed t-test. The 5 consecutive stimulation sessions as shown in (b) are averaged within individual mice.
(i) Comparison of changes in number of licks (Left, Δ# Licks, Before - After) and reaction time (Right, ΔRT) between baseline (n = 20) and stimulation (n = 20) sessions for the test group. Δ# Licks, Baseline vs. Stimulation: 1.48 ± 0.37 vs. 1.10 ± 0.33, p = 0.49, rank sum = 425; ΔRT, Baseline vs. Stimulation: 0.06 ± 0.02 vs. 0.01 ± 0.02 s, p = 0.13, rank sum = 455, two-tailed Wilcoxon rank-sum test. For stimulation sessions, the change (Δ) was calculated by subtracting the variable (# licks or RT) quantified in hit trials during optical stimulation trials from the variable quantified in hit trials during the last 50 trials of the previous block (i.e., no optical stimulation). For baseline sessions, no optical stimulation was delivered, and the change (Δ) was calculated by subtracting the variable quantified in hit trials during the first 50 trials following the rule change from the variable quantified in hit trials during the last 50 trials of the previous block.
(j) Same as in (h) but for the control group. Left: Changes in number of licks, Baseline vs. Stimulation: 1.53 ± 0.37 vs. 0.81 ± 0.28, p = 0.17, rank sum = 266. Right: Changes in reaction time, Baseline vs. Stimulation: 0.06 ± 0.02 vs. 0.06 ± 0.02 s, p = 0.80, rank sum = 239, two-tailed Wilcoxon rank-sum test.

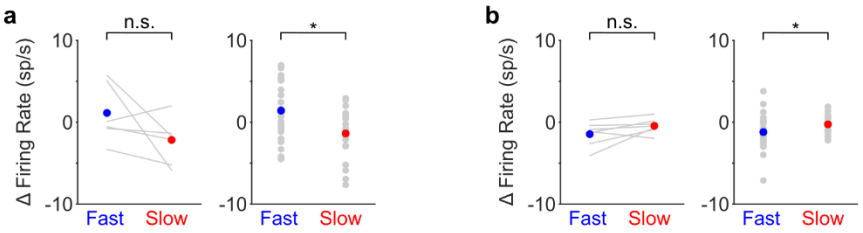

Figure S5. Quantifying LC response to whisker stimulation and auditory tone during behavior

(a) Changes in LC responses to whisker stimulation (After - Before) in hit trials during fast and slow switch blocks (Left: paired Fast vs. Slow: 1.14 ± 1.24 vs. -2.16 ± 1.01 spikes/s, p = 0.14, n = 6, two-tailed t-test; Right: unpaired Fast vs. Slow: 1.41 ± 0.63 vs. -1.37 ± 0.66 spikes/s, p = 0.012, 26 fast and 21 slow switch blocks, two-tailed Wilcoxon rank-sum test). LC responses to whisker stimulation were quantified in a 100-ms window beginning at stimulus onset, subtracted from LC activity quantified in a 0.5-s baseline window ending at stimulus onset. There were no hit trials during the Before period for 2 blocks from the original full dataset (49 blocks), and they were removed from this analysis. 1 block was included in the original paired analysis (7 fast-slow pairs), so the associated pair was removed from this analysis.

(b) The same as in (a) but for LC responses to the auditory tone (Left: paired Fast vs. Slow: -1.45 ± 0.55 vs. -0.44 ± 0.34 spikes/s, p = 0.11, n = 7, two-tailed t-test; Right: unpaired Fast (n = 28) vs. Slow (n = 21): -1.21 ± 0.38 vs. -0.26 ± 0.27 spikes/s, p = 0.042, two-tailed Wilcoxon rank-sum test). Tone responses were quantified in a 300-ms window beginning at tone onset, subtracted from LC activity quantified in a 0.5-s baseline window ending at tone onset.