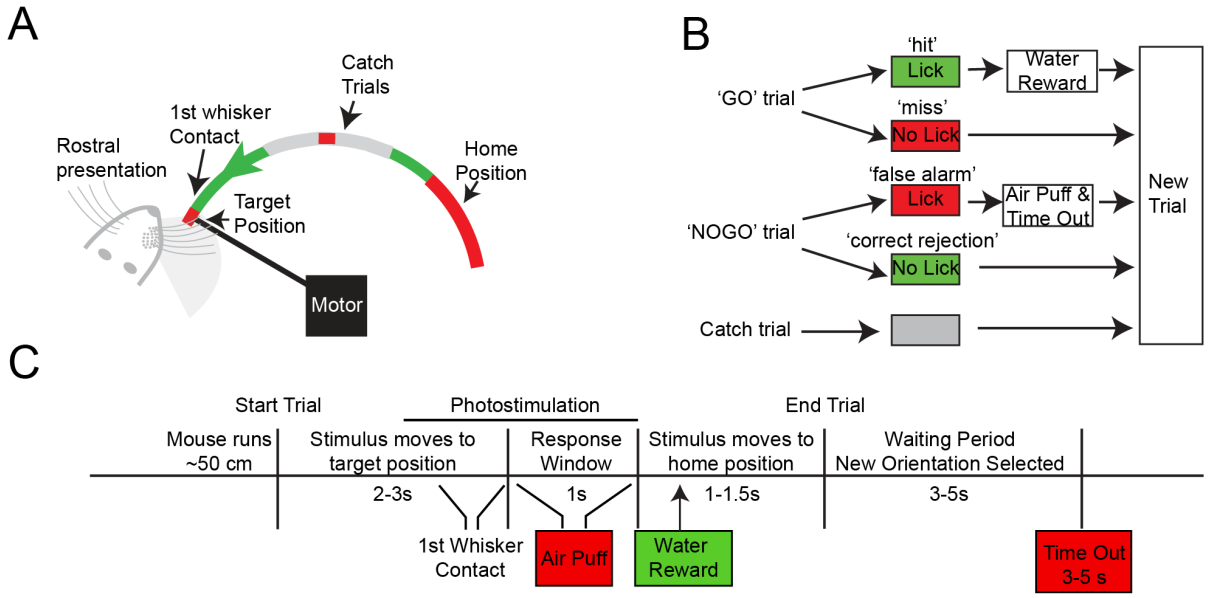
A
Catch Trials
1st whisker Contact
Rostral presentation
Home Position
Target Position
Motor
B
'hit'
Lick
Water Reward
'GO' trial
'miss'
No Lick
'false alarm'
Lick
Air Puff & Time Out
New Trial
'NOGO' trial
'correct rejection'
No Lick
Catch trial
C
Start Trial
Photostimulation
End Trial
Mouse runs ~50 cm
Stimulus moves to target position
Response Window
Stimulus moves to home position
Waiting Period New Orientation Selected
2-3s
1s
1-1.5s
3-5s
1st Whisker Contact
Air Puff
Water Reward
Time Out 3-5 s
D
Anticipatory Licking > 70%?
1Ori auto
Yes
No
Performance >70%?
1Ori
Yes
No
& 10% Catch
Performance >70%?
2Ori
Yes
No
& 10% Catch
d' >1.5?
8Ori
Yes
No
& 10% Catch
Experimentation
E
Mouse 1
Mouse 2
Mouse 3
Mouse 4
Mouse 5
Mouse 6
Mouse 7
Mouse 8
Moving Fraction Correct
Trial number
1Ori auto
1Ori
2Ori
8Ori
d'
Session Number
F
Reverse contingency
'NOGO'
0°
'GO'
+45°
-45°
Lick Probability
Original
Reverse
Stim. Orientation (°)
C

Figure S1. Orientation discrimination task design, Related to Figure 1

A. Schematic of behavioral task. The stimulus bar is presented rostrally to the mouses right whisker field. Mice can contact the bar as it travels into the 'target position'. For catch trials, the stimulus bar is held outside the whisker field. The 'home position' is jittered on each trial (red area).
B. GO/NOGO trial structure.
C. Timeline of trial structure.
D. Block diagram of progressive training paradigm.
E. *Left:* Moving average performance over training for 8 example mice. Dashed line: 70% correct performance. *Right:* d' over each session for the corresponding mice, dashed line: 1.5 d'. Colored lines represent training stage.
F. Performance on reverse GO/NOGO contingency with a full whisker pad (*top*: schematic, *bottom*: psychometric curve of performance (n=5 mice, averages from individual mice (gray); mean ± SEM and fit (black) and for comparison original contingency data as in Figure 1E (red)). Dashed lines represent midpoints for 0° orientation and 0.5 lick probability.

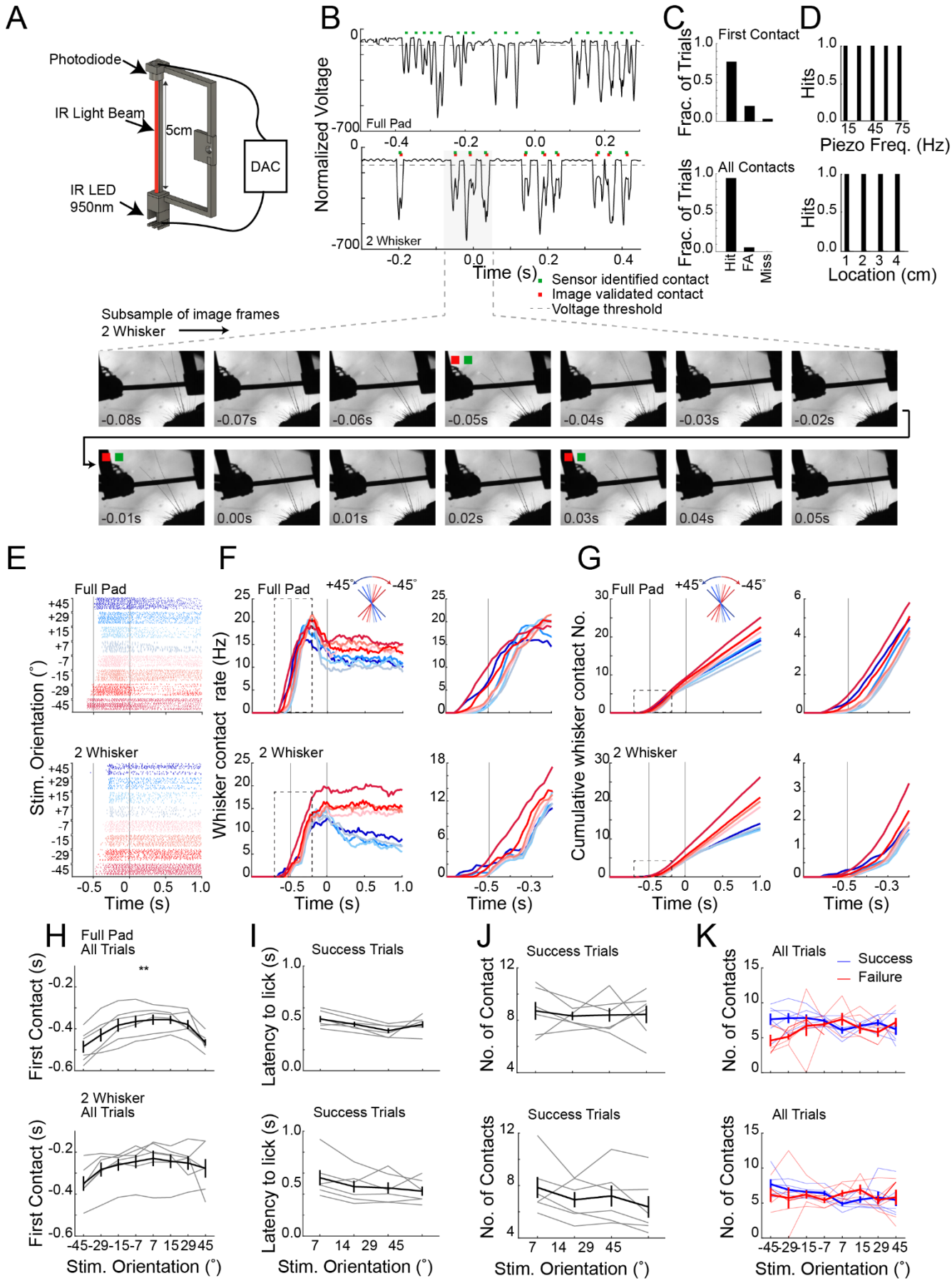
A
Photodiode
IR Light Beam
5cm
IR LED
950nm
DAC
B
Normalized Voltage
Full Pad
2 Whisker
Time (s)
Sensor identified contact
Image validated contact
Voltage threshold
C
First Contact
All Contacts
Frac. of Trials
Hit
FA
Miss
D
Hits
Piezo Freq. (Hz)
Location (cm)
Subsample of image frames
2 Whisker
-0.08s
-0.07s
-0.06s
-0.05s
-0.04s
-0.03s
-0.02s
-0.01s
0.00s
0.01s
0.02s
0.03s
0.04s
0.05s
E
Full Pad
2 Whisker
Stim. Orientation (°)
Time (s)
F
Whisker contact rate (Hz)
+45°
-45°
G
Cumulative whisker contact No.
H
Full Pad
All Trials
First Contact (s)
2 Whisker
All Trials
Stim. Orientation (°)
I
Success Trials
Latency to lick (s)
J
No. of Contact
No. of Contacts
K
All Trials
Success
Failure

Figure S2. Identification of whisker contacts during the task, Related to Figure 1

A. Schematic of stimulus bar with IR LED beam and photodiode for touch detection.
B. Normalized and smoothed signal from photodiode for an example trial of a mouse performing the task with full whisker pad (*top*) and two whiskers (*bottom*); Voltage deflection threshold (3 SD from baseline, grey dashed line). Photodiode detected whisker contacts (green square) and for the two-whisker trace visually identified whisker contacts (red square). *Below*: a subset of high-speed imaging frames from the two-whisker data.
C. Validation of the touch detector with simultaneous high-speed imaging for mice trimmed to two whiskers. Validation for first whisker contact (*top*) and subsequent contacts (*bottom*) (n=3 mice, 3 sessions, 30 trials for first contact, 76 trials for subsequent).
D. Whisker tethered piezo validation at different frequencies (*top*), and different contact points along the stimulus bar (*bottom*) (n= 60 repetitions at each condition).
E. Raster plot for an example mouse with full whisker pad (*top*) and two-whiskers (*bottom*) showing detected whisker contacts (colored tick marks) sorted by stimulus orientation. Gray lines indicate time points –0.5 and 0 seconds for visual guide.
F. Whisker contact rate for full pad (*top*) and two-whisker (*bottom*) sessions separated by stimulus orientation. Box represents zoomed in panel shown to the right.
G. As in F but cumulative whisker contact number over time.
H. Time of first whisker contact for all trials from full whisker pad data (*top*) and two-whisker data (*bottom*) over all stimulus orientations (n=3 mice, 6 sessions, **p<0.01, ANOVA).
I. Latency from first whisker contact to lick response for success trials for full pad data (*top*) and two-whisker data (*bottom*) (n=3 mice, 6 sessions).
J. Number of whisker contacts before lick response on success trials for full pad data (*top*) and two-whisker data (*bottom*) (n=3 mice, 6 sessions).
K. Number of whisker contacts within 0.3 seconds from first contact for all trials separated into success (blue) and failure (red) trials for full whisker data (*top*) and two-whisker data (*bottom*, individual sessions shown in lighter colors and group mean ± SEM, solid line; n=3 mice, 6 sessions).

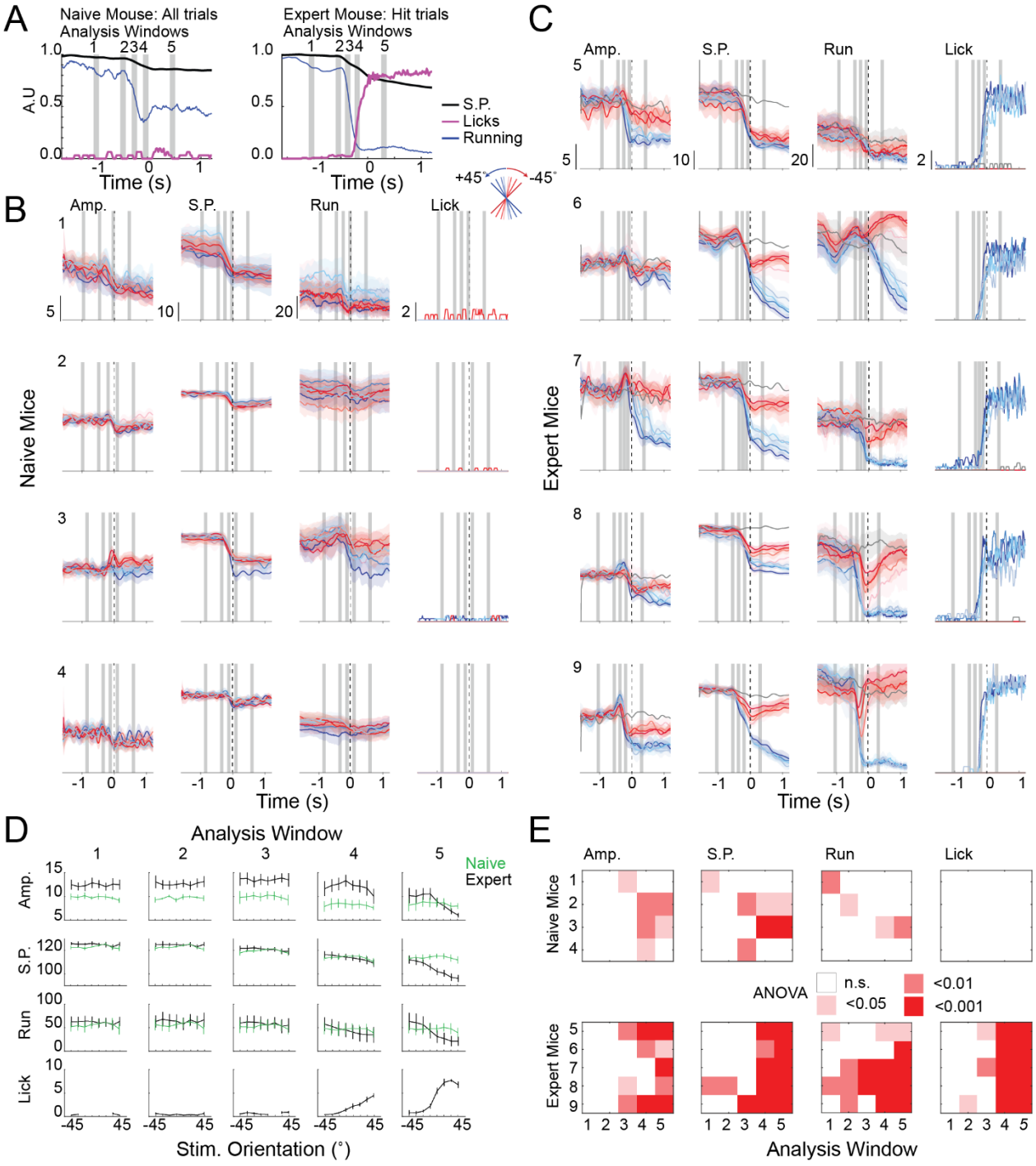
A
Naive Mouse: All trials
Analysis Windows
Expert Mouse: Hit trials
Analysis Windows
A.U
Time (s)
S.P.
Licks
Running
+45°
-45°
B
Amp.
S.P.
Run
Lick
Naive Mice
Time (s)
C
Expert Mice
Time (s)
D
Analysis Window
Naive
Expert
Stim. Orientation (°)
E
Naive Mice
Expert Mice
ANOVA
n.s.
<0.05
<0.01
<0.001
Analysis Window

Figure S3. Learning induced changes in running, whisking, and licking behavior, Related to Figure 1

A. Example naive (*left*) and expert (*right*) mouse average run speed (blue), whisker set point (S.P.; black), lick rate (magenta) for all trials (naive mice) and hit trials (expert mice). Vertical gray lines represent analysis windows; (#1) baseline, (#2-4) start, middle and end of sampling window, (#5) response window. See methods.
B. Behavior responses from all trials for naive mice separated by stimulus orientations. Average whisker amplitude (Amp.), whisker set point (S.P.), running velocity (Run) and lick rate (Lick) ± confidence interval (n=4 mice). Individual colors represent stimulus orientation. Vertical gray lines represent each analysis window as in A. Dashed black line represents time 0 corresponding to the start of the response period.
C. As in B but success trials for expert mouse (n=5 mice).
D. Mean measurements during each analysis window across each stimulus orientation for naive (green) and expert (black) mice.
E. Statistical test of significance (ANOVA) between each orientation, for each mouse, for each analysis window and for each behavior for naive (*top*) and expert (*bottom*) mice (white, P=ns; light pink=*P<0.05, light red **P<0.01, red ***P<0.001.

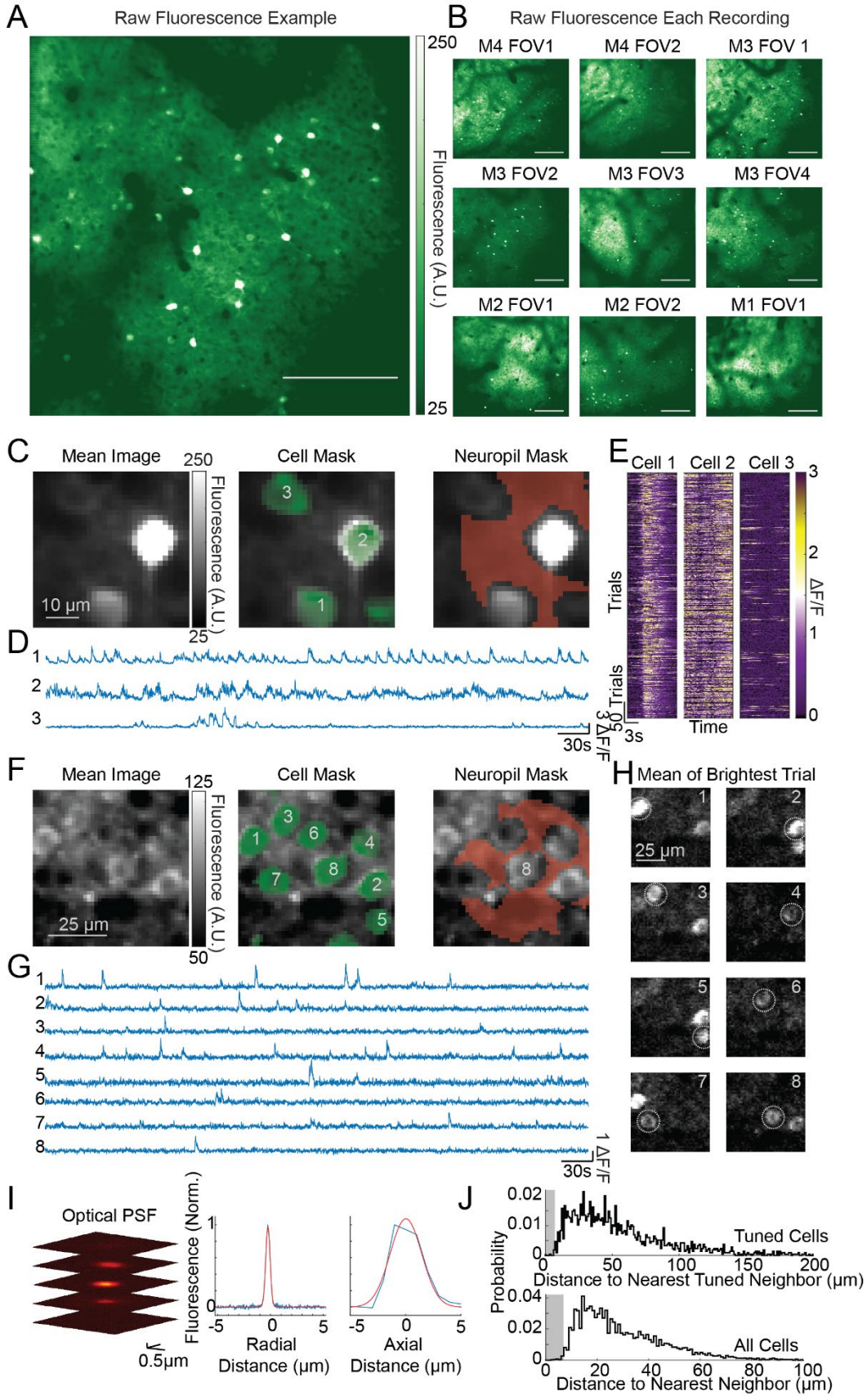
A
Raw Fluorescence Example
250
Fluorescence (A.U.)
25
B
Raw Fluorescence Each Recording
M4 FOV1
M4 FOV2
M3 FOV 1
M3 FOV2
M3 FOV3
M3 FOV4
M2 FOV1
M2 FOV2
M1 FOV1
C
Mean Image
250
Fluorescence (A.U.)
25
10 µm
Cell Mask
Neuropil Mask
D
1
2
3
30s
3 ΔF/F
E
Cell 1
Cell 2
Cell 3
3
2
ΔF/F
1
0
Trials
Trials
0s
3s
Time
F
Mean Image
125
Fluorescence (A.U.)
50
25 µm
Cell Mask
Neuropil Mask
G
1
2
3
4
5
6
7
8
30s
1 ΔF/F
H
Mean of Brightest Trial
25 µm
I
Optical PSF
0.5µm
Fluorescence (Norm.)
1
0
-5
0
5
Radial
Distance (µm)
-5
0
5
Axial
Distance (µm)
J
0.02
0.01
0
Probability
Tuned Cells
0
50
100
150
200
Distance to Nearest Tuned Neighbor (µm)
0.04
0.02
0
All Cells
0
20
40
60
80
100
Distance to Nearest Neighbor (µm)

Figure S4. Imaging quality and source separation, Related to Figure 4

A. Motion corrected mean raw fluorescence image from a single imaging plane from an example mouse. Scale Bar 200μm.
B. As in A but from a single plane from each mouse (M) and session included in the analysis.
C. Motion corrected mean image of raw fluorescence (*left*), weighted pixel cell masks are overlaid on the mean image (*middle*), the region that the neuropil signal is extracted from for cell #2 (*right*).
D. Example ΔF/F traces from each cell within the FOV in C.
E. ΔF/F responses of each cell in C, aligned to the start of a trial, all trials from one recording session are presented.
F. As in C, but from a region that appeared much dimmer in the mean image.
G. As in D.
H. Average fluorescence from the trial when each cell in F is most active. White circle denotes the cell of interest.
I. Optical point spread function (PSF) of our imaging system, image of a 200nm fluorescent bead imaged at high zoom and planes spaced by 1μm (*letf*). The fluorescence profile over radial (*middle*) and axial (*right*) distance, raw data (blue) is fit with a gaussian (red). FWHM radial 0.49μm, axial 3.4μm.
J. Spatial distribution of nearest tuned (*top*) or any (*bottom*) neighboring cells. Gray area represents the average width of an ROI.

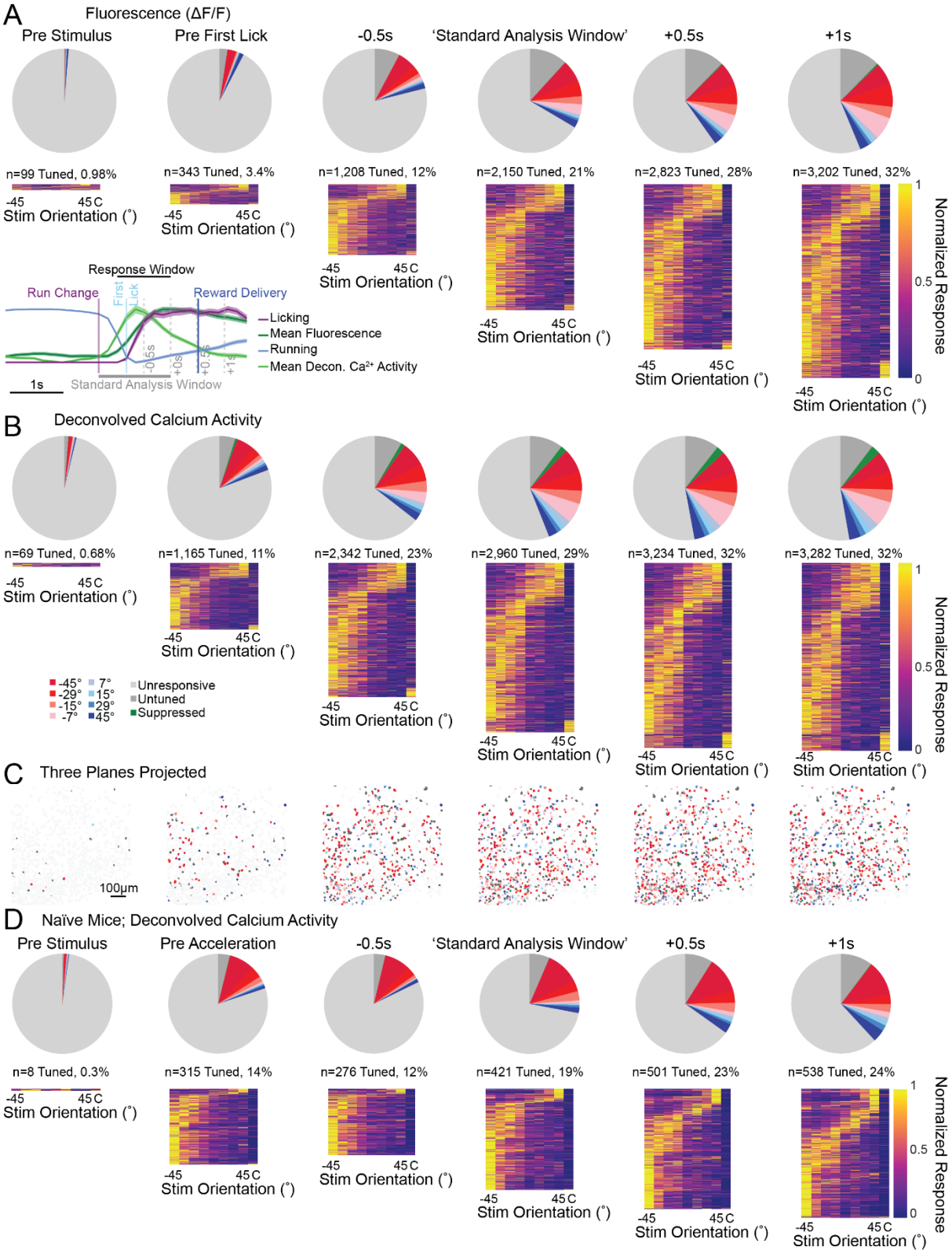
A
Fluorescence (ΔF/F)
Pre Stimulus
Pre First Lick
-0.5s
'Standard Analysis Window'
+0.5s
+1s
n=99 Tuned, 0.98%
n=343 Tuned, 3.4%
n=1,208 Tuned, 12%
n=2,150 Tuned, 21%
n=2,823 Tuned, 28%
n=3,202 Tuned, 32%
-45
45
C
Stim Orientation (˚)
Response Window
Run Change
First Lick
Reward Delivery
-0.5s
+0s
+0.5s
+1s
1s
Standard Analysis Window
Licking
Mean Fluorescence
Running
Mean Decon. Ca²⁺ Activity
1
0.5
0
Normalized Response
B
Deconvolved Calcium Activity
n=69 Tuned, 0.68%
n=1,165 Tuned, 11%
n=2,342 Tuned, 23%
n=2,960 Tuned, 29%
n=3,234 Tuned, 32%
n=3,282 Tuned, 32%
-45°
-29°
-15°
-7°
7°
15°
29°
45°
Unresponsive
Untuned
Suppressed
C
Three Planes Projected
100μm
D
Naïve Mice; Deconvolved Calcium Activity
Pre Stimulus
Pre Acceleration
-0.5s
'Standard Analysis Window'
+0.5s
+1s
n=8 Tuned, 0.3%
n=315 Tuned, 14%
n=276 Tuned, 12%
n=421 Tuned, 19%
n=501 Tuned, 23%
n=538 Tuned, 24%

Figure S5. Establishing the window for calcium response analysis, Related to Figure 4

A. *Top:* The proportion of touch responsive or tuned cell for each analysis window (see Methods). The color (blue to red) indicates preferred orientation of neurons, (dark gray) untuned neurons, (green) suppressed neurons, and (light gray) unresponsive neurons as in Figure 4 (n=4 mice, 9 recordings, 10,140 cells). *Bottom*: The cross-validated tuning curves for all tuned neurons for each analysis window. Tuning curves are normalized and sorted by the preferred stimulus of half the data that is not shown. *Inset:* Trace of a representative mouse's run speed (all trial types, blue), lick rate (Hit trials, purple), mean calcium fluorescence (mean z-scored ΔF/F, dark green), or deconvolved calcium response (mean z-scored deconvolved calcium activity, light green). Standard analysis window (gray bar), run deviation frame, aka the estimated time of first contact (purple), response window (black line), beginning of the lick response (in hit trials, light blue), time of reward delivery (dark blue).
B. As in (A) but analyzed from deconvolved calcium activity data.
C. Stimulus preference maps of all neurons recorded in a single recording session as a function of the analysis window; 3 imaging planes are superimposed. Significantly tuned cells are color coded by their preferred orientation (red to blue), untuned but touch-responsive cells (dark gray), and unresponsive cells (light gray).
D. As in (A) but analyzed from untrained, naive (n=2 mice, 3 recordings, 2,200 cells). The 'pre-lick' period is now a window that ends when the mouse begins to accelerate after stimulus presentation.

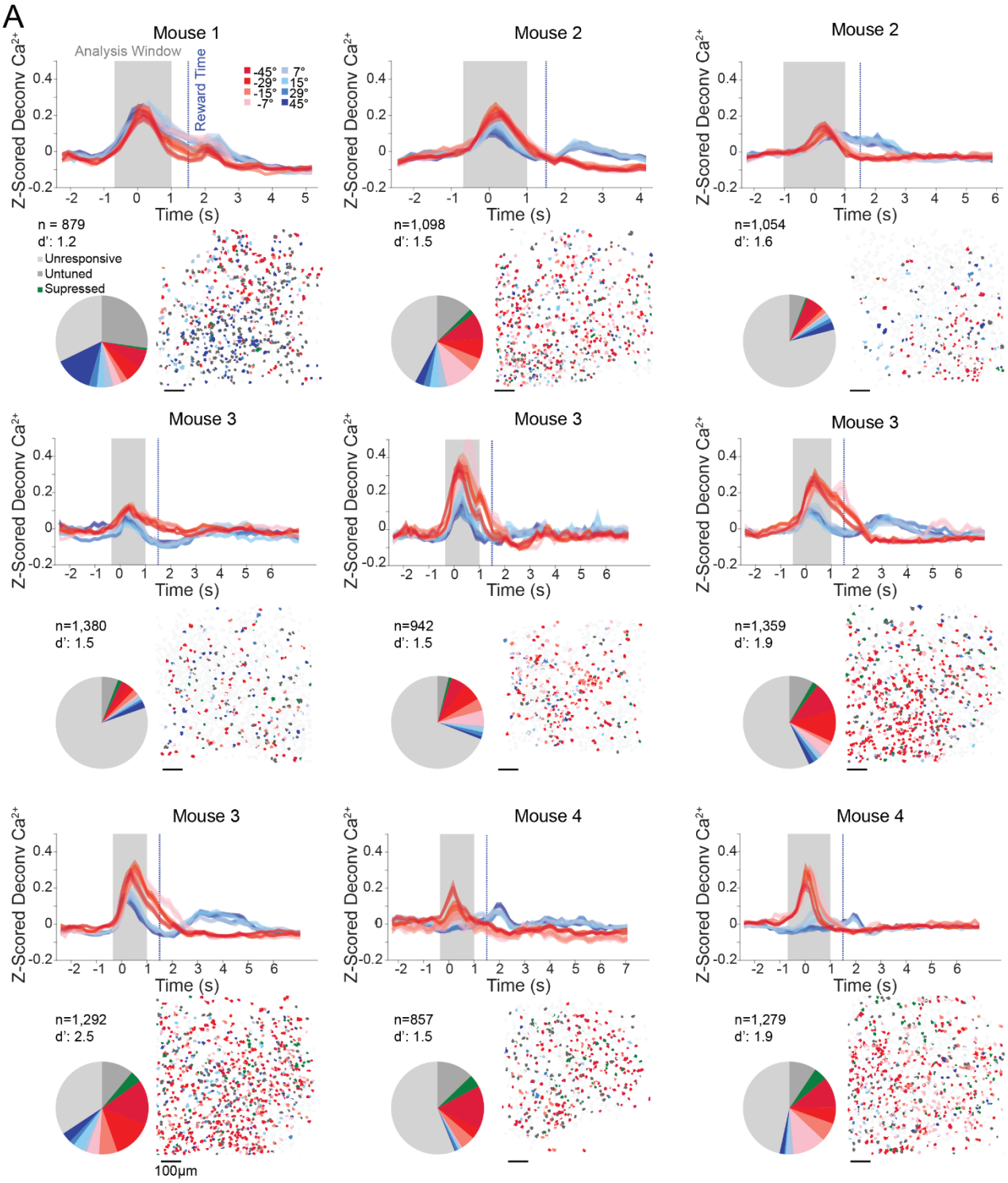
A
Mouse 1
Analysis Window
Reward Time
-45°
-29°
-15°
-7°
7°
15°
29°
45°
Z-Scored Deconv Ca2+
Time (s)
n = 879
d': 1.2
Unresponsive
Untuned
Supressed
Mouse 2
n=1,098
d': 1.5
Mouse 2
n=1,054
d': 1.6
Mouse 3
n=1,380
d': 1.5
Mouse 3
n=942
d': 1.5
Mouse 3
n=1,359
d': 1.9
Mouse 3
n=1,292
d': 2.5
100µm
Mouse 4
n=857
d': 1.5
Mouse 4
n=1,279
d': 1.9

Figure S6. Calcium responses from each field of view, Related to Figure 4

A. For each recording, *top,* mean (±95% confidence interval) population deconvolved calcium activity for each presented orientation (see inset). Gray window: the analysis window used see Figure S5). Blue dotted line: the time of reward delivery. Inset: the number of detected neurons, and d' recorded during this session. *Bottom left*: Pie chart showing the relative percent of neurons that were unresponsive, untuned, suppressed, or tuned as in Figure 4C. *Bottom right*: stimulus preference map of all neurons recorded in a single recording session; 3 imaging planes are superimposed as in Figure 4F (Scalebar 100μm). The example data in Figure 4 comes from Mouse 1.

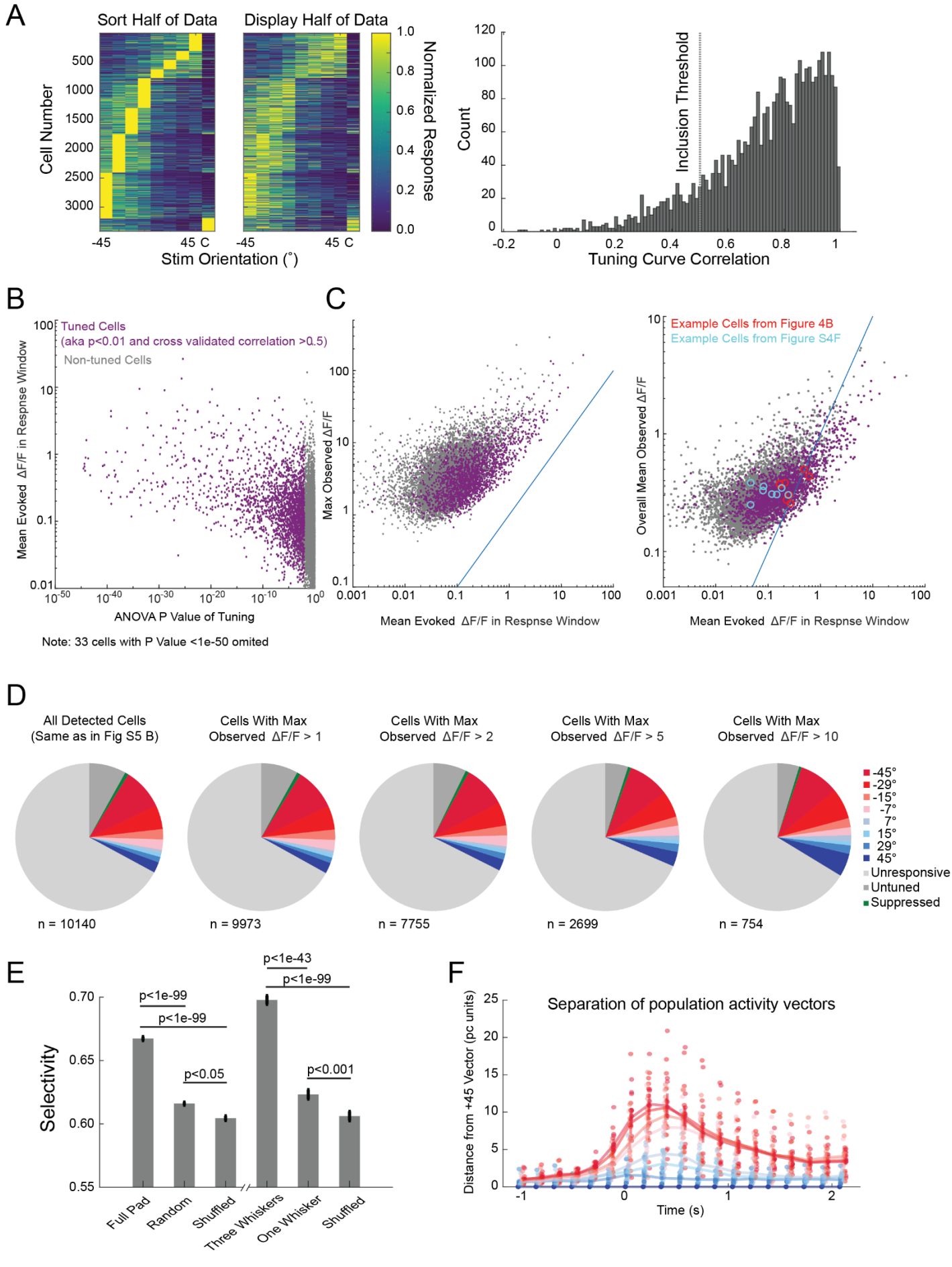
A
Sort Half of Data
Display Half of Data
Cell Number
Normalized Response
Stim Orientation (˚)
Count
Inclusion Threshold
Tuning Curve Correlation
B
Tuned Cells
(aka p<0.01 and cross validated correlation >0.5)
Non-tuned Cells
Mean Evoked ΔF/F in Respnse Window
ANOVA P Value of Tuning
Note: 33 cells with P Value <1e-50 omited
C
Max Observed ΔF/F
Mean Evoked ΔF/F in Respnse Window
Example Cells from Figure 4B
Example Cells from Figure S4F
Overall Mean Observed ΔF/F
D
All Detected Cells
(Same as in Fig S5 B)
Cells With Max
Observed ΔF/F > 1
Cells With Max
Observed ΔF/F > 2
Cells With Max
Observed ΔF/F > 5
Cells With Max
Observed ΔF/F > 10
n = 10140
n = 9973
n = 7755
n = 2699
n = 754
-45°
-29°
-15°
-7°
7°
15°
29°
45°
Unresponsive
Untuned
Suppressed
E
Selectivity
p<1e-99
p<1e-99
p<0.05
p<1e-43
p<1e-99
p<0.001
Full Pad
Random
Shuffled
Three Whiskers
One Whisker
Shuffled
F
Separation of population activity vectors
Distance from +45 Vector (pc units)
Time (s)

Figure S7. Population metrics of calcium imaging data, Related to Figure 4

A. Explanation of cross-validated tuning curves. *Left*: data was split in half and assigned to a sort half (*left*) or a display half (*right*). Tuning curves from cells determined to be tuned by the complete dataset (ANOVA <0.01 on all trial types excluding catch) were calculated for each half and sorted based on the response in the sort half. *Right*: histogram of the correlation between the tuning curves of each cell determined by the sorted half or the displayed half of the data. Only tuning curves from the displayed half that passed the inclusion threshold correlation of 0.5 are displayed in cross validated tuning plots. 2960/3416 (87%) tuned cells (defined as ANOVA <0.01 between orientations) passed this threshold in the standard analysis (Figure 4D).
B. Mean evoked ΔF/F during response window and corresponding ANOVA P value for all cells. Color coded by tuned (purple) and non-tuned (gray). Note: tuning requires both a low P value, and a high cross-validation correlation.
C. (*Left)* Maximum ΔF/F observed at any point during the recording session and mean ΔF/F in the response window for all cells, separated by tuned (purple) and non-tuned (gray). (*Right)* Overall mean ΔF/F response during entire recording vs the mean ΔF/F recorded during the analysis window. Example cells from Figure 4B (red) and S4F (blue) are circled.
D. Pie chart showing relative fractions of tuning for all detected cells (*left*) and with increasing restrictive criteria of ΔF/F, i.e. if we only include higher signal cells how does our data change.
E. Selectivity index computed for all detected neurons from normal experiments (mean ± SEM) compared to similar metrics created by random values or shuffled data, and from neurons from the trimming experiments. P values are rank sum comparisons between populations.
F. Euclidean distance of population activity across the first 3 principal components measured in each frame as the distance from the +45° vector to each of the other orientations. Faint dots denote each recording's average, solid line denotes mean ± SEM.

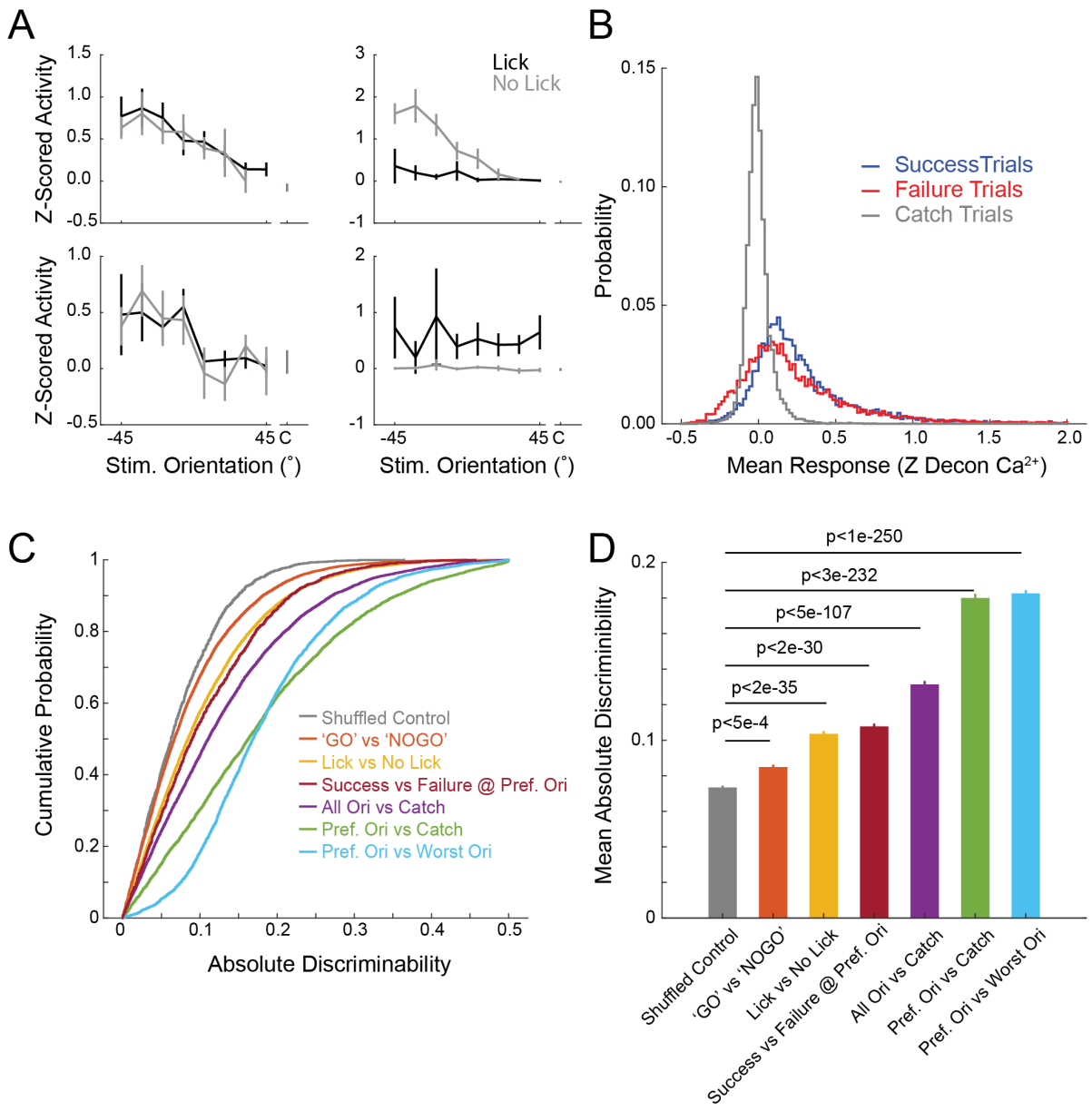
A
Z-Scored Activity
Lick
No Lick
Stim. Orientation (˚)
B
Probability
SuccessTrials
Failure Trials
Catch Trials
Mean Response (Z Decon Ca2+)
C
Cumulative Probability
Shuffled Control
'GO' vs 'NOGO'
Lick vs No Lick
Success vs Failure @ Pref. Ori
All Ori vs Catch
Pref. Ori vs Catch
Pref. Ori vs Worst Ori
Absolute Discriminability
D
Mean Absolute Discriminibility
p<1e-250
p<3e-232
p<5e-107
p<2e-30
p<2e-35
p<5e-4
Shuffled Control
'GO' vs 'NOGO'
Lick vs No Lick
Success vs Failure @ Pref. Ori
All Ori vs Catch
Pref. Ori vs Catch
Pref. Ori vs Worst Ori

Figure S8. Modulation of neural activity by behavioral responses, Related to Figure 4

A. Tuning curve of 4 example neurons separated between lick and no lick trials (mean ± confidence interval).
B. Histogram of responses to preferred orientation during success (blue) and failure (red) trials, and the corresponding cells' responses during catch trials (gray). Only cells with at least one failure trial in the preferred condition were included (n=8,558 cells).
C. Cumulative probability plots of the discriminability calculated from the response of each neuron comparing different conditions. Analysis was restricted to the four most vertical angles (±15°, ±7°), comparisons with fewer than 10 responses in each category were excluded. If a comparison involved the preferred orientation of a cell, that orientation was derived from the full 8 orientation set, but only cells with a preference in the middle 4 orientations were used. Shuffled data matches the trial counts of the fewest trials of any of the comparisons (shuffle n=1687, GO v NOGO n=10,140, lick v no lick n=10,140, success v failure (at preferred orientation) n=1,687, all orientations v catch n=9198, preferred orientation v catch n=4,131, preferred orientation v worst orientation n=4,606 cells).
D. The mean ± SEM of the discriminability values displayed in C, and the P value (rank sum test) of the difference between this distribution and the shuffled control.

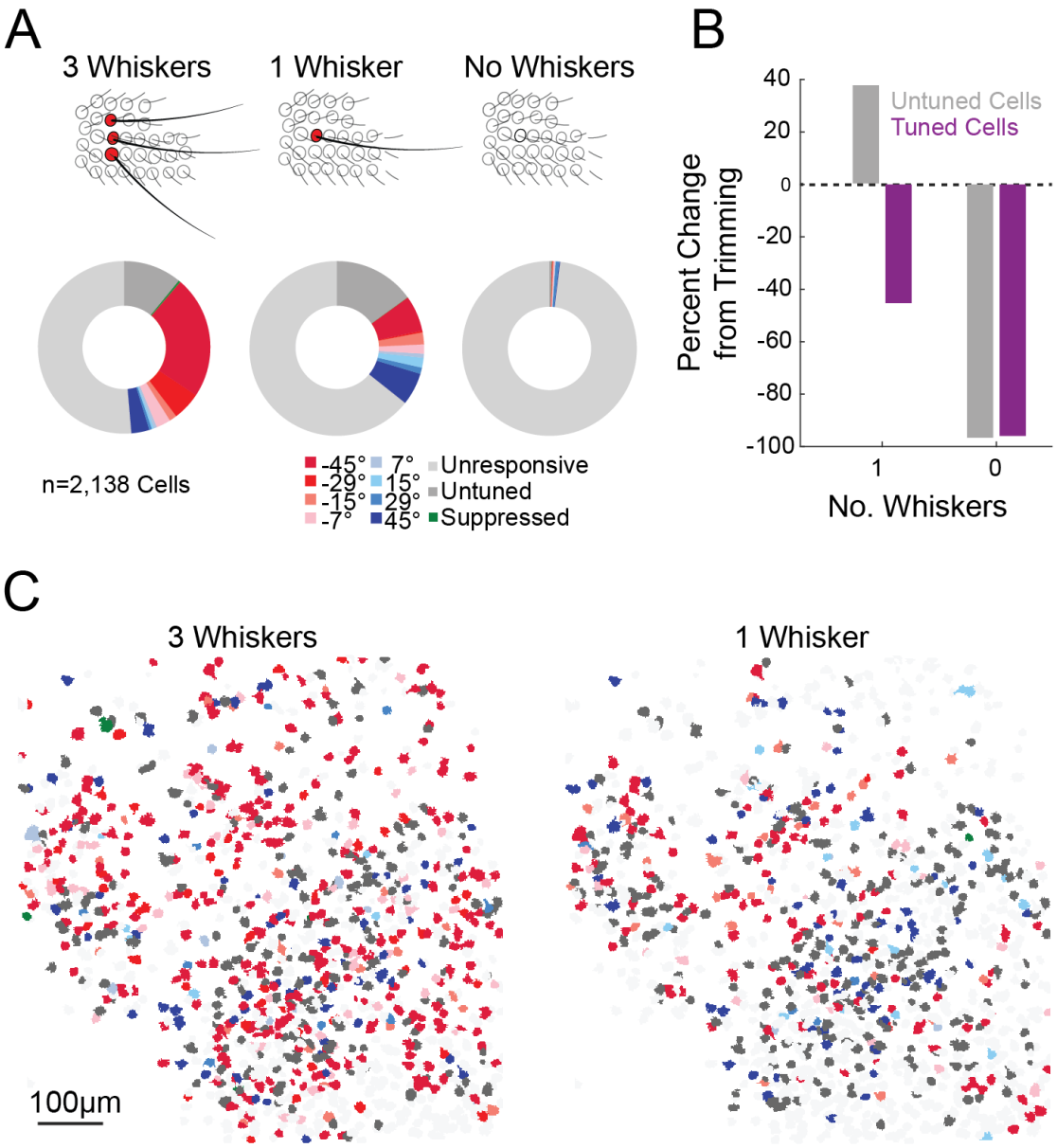
A
3 Whiskers
1 Whisker
No Whiskers
n=2,138 Cells
-45°
-29°
-15°
-7°
7°
15°
29°
45°
Unresponsive
Untuned
Suppressed
B
Untuned Cells
Tuned Cells
Percent Change from Trimming
40
20
0
-20
-40
-60
-80
-100
1
0
No. Whiskers
C
3 Whiskers
1 Whisker
100µm

Figure S9. Whisker trimming reduces the number of orientation-tuned cells, Related to Figure 4

A. *Top*: schematic of the trimming experiment. *Bottom*: Fraction of neurons that were significantly tuned or untuned but touch responsive in the three-whisker, one-whisker, or no whisker condition (n= 2 mice, 2,138 cells).
B. Relative change in the number of cells that could be classified as touch responsive but untuned ('untuned', gray) or significantly tuned to any orientation ('tuned cells', magenta) for the one and no whisker conditions relative to the three-whisker condition. Maps of the locations of the recorded neurons (three planes superimposed) colored according to their preferred stimuli for example mouse, with three (*left*) or one (*right*) whisker remaining.