Cell Reports, Volume *43*

Supplemental information

Exploration biases forelimb reaching strategies

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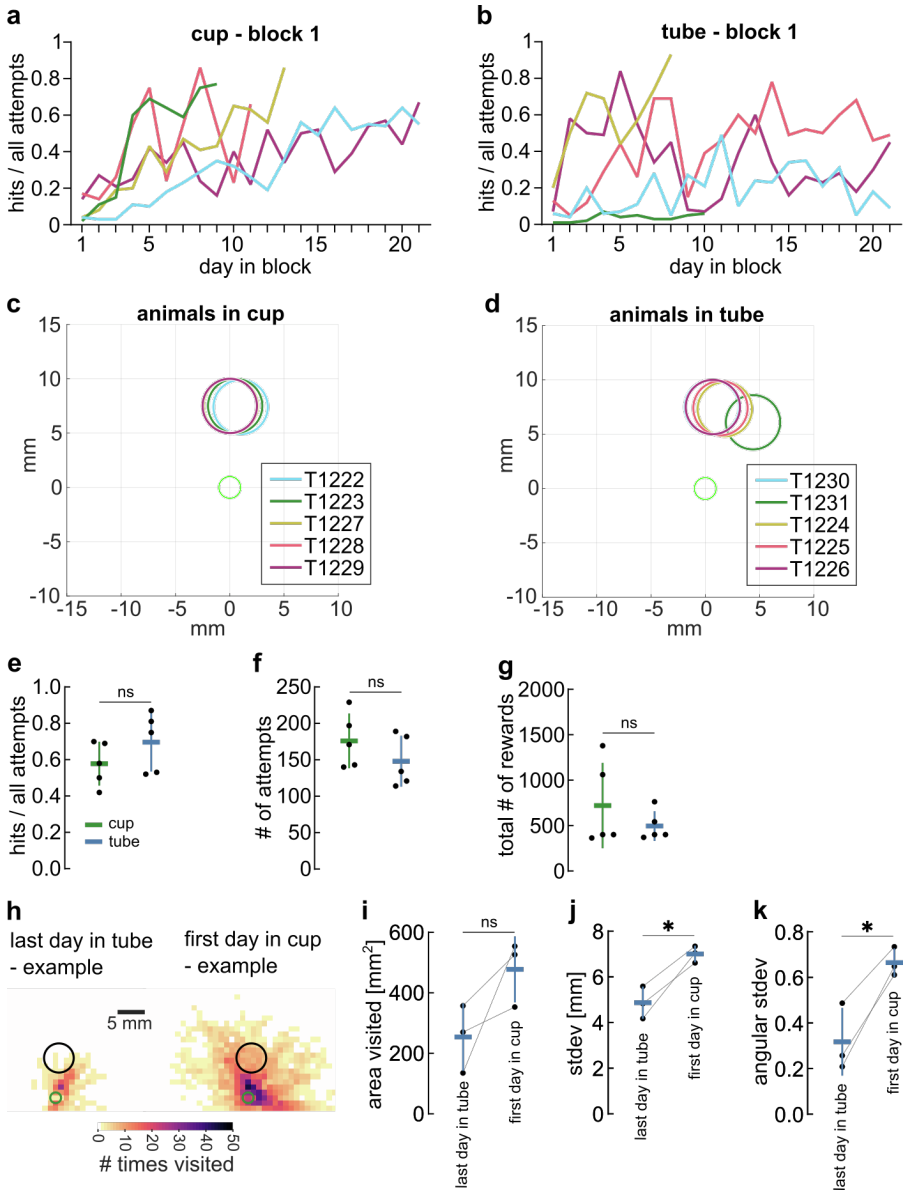

Figure S1: Behavior of animals trained in the cup and tube, Related to Figure 1.
(A-D) Colors indicate animals trained in the same joystick setup. **(A)** Hit ratio of animals trained in the cup for all sessions of block 1 until performance or termination criterion was met. **(B)** Same as (A) for animals trained in the tube. **(C)** Location of targets for animals trained in the cup, x/y: 0.32/7.48 ± 0.43/0.04 mm. **(D)** Location of targets for animals trained in the tube, x/y: 1.78/7.16 ± 1.55/0.60 mm, unpaired t-test (cup vs. tube), x: $t(8) = 2.03$, $p > 0.05$, y: $t(8) = 1.19$, $p > 0.05$. **(E)** Hit ratio on last day of pre-training for animals trained in the cup and the tube (unpaired t-test: $t(8) = 1.31$, $p > 0.05$, $n = 5$). **(F)** Number of attempts on last day of pre-training for animals trained in the cup and the tube (unpaired t-test: $t(8) = 1.22$, $p > 0.05$, $n = 5$). **(G)** Number of total rewards received during all of pre-training (phase 2) by animals trained in the cup and the tube (unpaired t-test: $t(8) = 1.01$, $p > 0.05$, $n = 5$). **(H)** Example heat maps showing the number of times a given 1 mm^2 bin of the workspace was visited during the last session in the tube and the first session in the cup of the same animal (green circle = start position, black circle = target). **(I)** Total area visited by all trajectories on the last day in the tube and the first day in the cup of the same animal (paired t-test: $t(2) = 2.22$, $p > 0.05$, $n = 3$). **(J)** Variability of the mean trajectory of all attempts on the last day in the tube and the first day in the cup of the same animal (paired t-test: $t(2) = 5.79$, $p < 0.05$, $n = 3$). **(K)** Variability of target entry vector direction on the last day in the tube and the first day in the cup of the same animal (paired t-test: $t(2) = 6.23$, $p < 0.05$, $n = 3$). Mean ± SD and single animals. *$p < 0.05$; ns, $p > 0.05$.

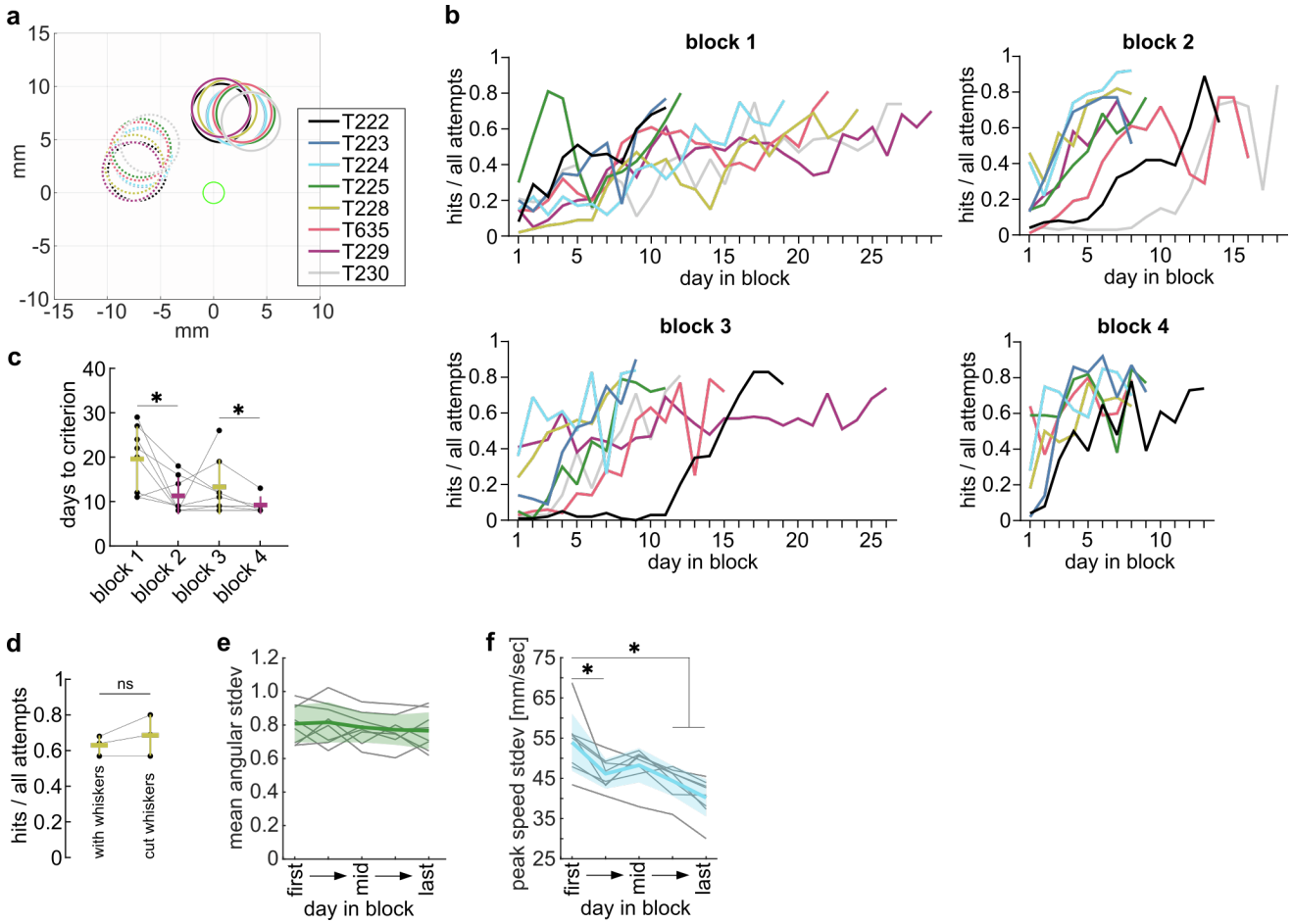

Figure S2: Main cohort targets and learning curves, Related to Figure 1/2.
(A) Location of targets for all animals of the main cohort, target 1 = solid circles, target 2 = dashed circles. **(B)** Hit ratio for all sessions until performance criterion for blocks 1-3 (n = 8) and block 4 (n = 6). **(C)** Number of days in each block to reach the performance criterion showing an overall target effect (Mixed-effects model, target: $F(1,7) = 8.62$, $p < 0.05$; Bonferroni's multiple comparison: block 1 vs 2: $t(7) = 2.96$, $p < 0.05$, block 3 vs 4: $t(5) = 3.35$, $p < 0.05$). **(D)** Hit ratio of animals that learned to hit target 1 with intact whiskers and on the day after whiskers were cut (paired t-test: $t(2) = 1.63$, $p > 0.05$, n = 3). **(E)** Mean spatial directional variability of full-length trajectories across blocks (one-way ANOVA, $F(2.7,18.6) = 0.97$, $p > 0.05$). **(F)** Variability of peak speed of hit trajectories across blocks (one-way ANOVA, $F(2.2,15.3) = 15.05$, $p < 0.01$). Asterisks show Dunnett's multiple comparisons between the first day and all other days of $p < 0.05$. Mean ± SD and single animals. *$p < 0.05$; ns, $p > 0.05$.

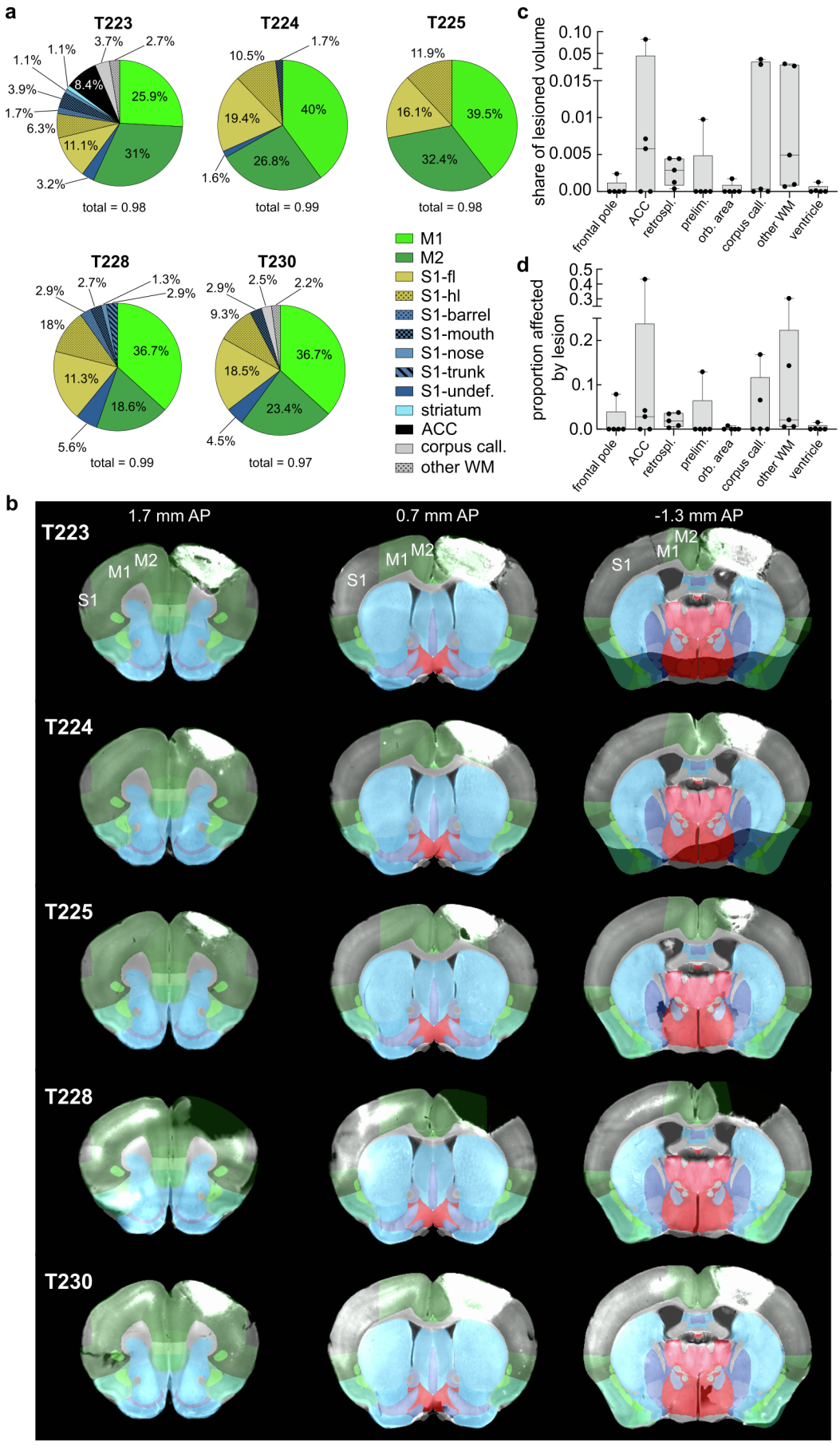
a
T223
T224
T225
T228
T230
25.9%
31%
11.1%
3.2%
6.3%
1.7%
3.9%
1.1%
1.1%
3.7%
2.7%
8.4%
total = 0.98
40%
26.8%
19.4%
1.6%
10.5%
1.7%
total = 0.99
39.5%
32.4%
16.1%
11.9%
total = 0.98
36.7%
18.6%
11.3%
5.6%
18%
2.9%
2.7%
1.3%
2.9%
total = 0.99
36.7%
23.4%
18.5%
4.5%
9.3%
2.9%
2.5%
2.2%
total = 0.97
M1
M2
S1-fl
S1-hl
S1-barrel
S1-mouth
S1-nose
S1-trunk
S1-undef.
striatum
ACC
corpus call.
other WM
c
share of lesioned volume
0.10
0.05
0.015
0.01
0.005
0.00
frontal pole
ACC
retrospl.
prelim.
orb. area
corpus call.
other WM
ventricle
d
proportion affected by lesion
0.5
0.4
0.3
0.2
0.1
0.0
frontal pole
ACC
retrospl.
prelim.
orb. area
corpus call.
other WM
ventricle
b
1.7 mm AP
0.7 mm AP
-1.3 mm AP
T223
T224
T225
T228
T230
M1
M2
S1

Figure S3: Stroke lesion quantification, Related to Figure 4.
(A) Pie charts for individual animals showing share of lesioned volume affecting different Allen Reference Brain Atlas areas. Only areas comprising > 1% of the total stroke lesion volume are shown. **(B)** Overlay of Allen Reference Brain Atlas at 3 different coronal planes (columns) with registered and aligned histological sections showing autofluorescence in grey scale for each animal (rows). T228 and T230 have fluorescent cells in the contralateral hemisphere from AAV injections. For T228 the lesioned tissue is missing as it washed off during section processing. **(C)** Relative share of total stroke volume that affected additional Allen Reference Brain Atlas areas. **(D)** Additional Allen Reference Brain Atlas areas that were affected by the stroke lesion showing the proportion of volume lesioned. Abbreviations. M1: primary motor cortex, M2: secondary motor cortex, S1-fl: primary sensory cortex – forelimb, S1-hl: primary sensory cortex – hindlimb, S1-barrel: primary sensory cortex – barrel, S1-mouth: primary sensory cortex – mouth, S1-nose: primary sensory cortex – nose, S1-trunk: primary sensory cortex – trunk, S1-undef.: primary sensory cortex – undefined, ACC: anterior cingulate cortex, retrospl.: retrosplenial, prelim.: prelimbic, orb. area.: orbital area, corpus call.: corpus callosum, WM: white matter. Box plots showing median, quartiles and min/max whiskers, as well as single animal data (black dots).

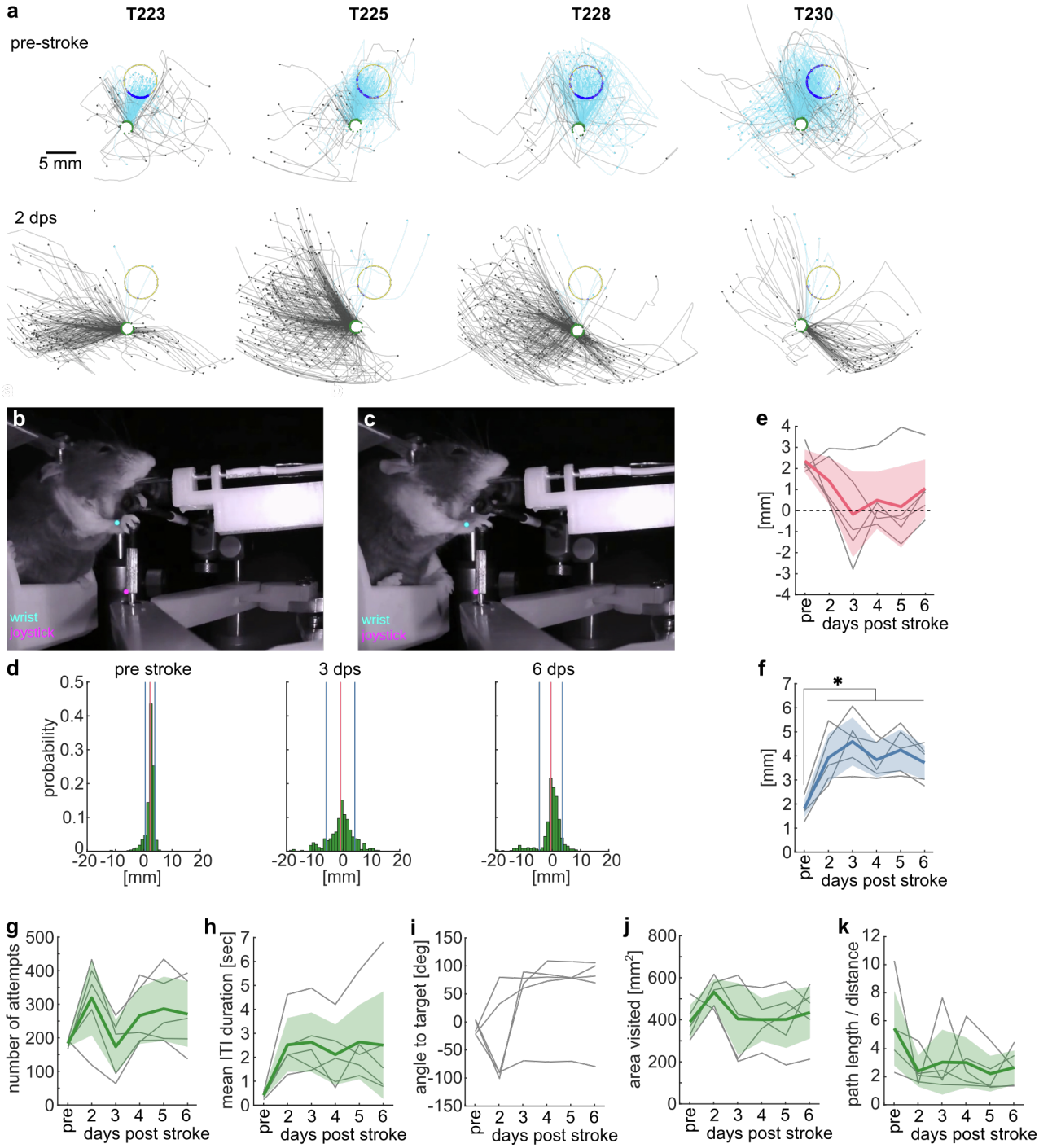

Figure S4: Additional behavior analysis after stroke, Related to Figure 4.
(A) Reach trajectories before and 2 days post stroke of the remaining 4 animals not shown in Figure 4E. **(B)** Example video frame showing wrist and joystick key points tracked using lightning pose in a pre-stroke session, (cyan = wrist, magenta = joystick). **(C)** Same as (B) but 2 days post stroke. **(D)** Example data from a single animal pre-stroke and 3 and 6 days post stroke showing histograms of the distance between the wrist and the joystick key points in the x-dimension of the video during active joystick movements. Red line = mean, blue lines = ± SD. **(E)** Mean x-dimension distance between the wrist and joystick for all animals and sessions. Positive values = the wrist is left of the joystick in the x-dimension of the video frame. Negative values = the wrist is right of the joystick in the x-dimension of the video frame (one-way ANOVA, $F(1.9,7.5) = 3.38$, $p > 0.05$). **(F)** Standard deviation of x-dimension distance between the wrist and joystick for all animals and sessions (one-way ANOVA, $F(2.7,10.7) = 14.59$, $p < 0.01$). **(G)** Total number of attempts made per session before and

after the stroke lesion (one-way ANOVA, $F(2.1,8.4) = 3.56$, $p > 0.05$). **(H)** Mean time between attempts before and after the stroke lesion (one-way ANOVA, $F(1.5, 5.9) = 4.91$, $p > 0.05$). **(I)** Initial direction angle difference to target showing medial and lateral deviation of single animals. **(J)** Total workspace area visited by all trajectories before and after stroke (one-way ANOVA, $F(2.0,8.0) = 1.40$, $p > 0.05$). **(K)** Mean tortuosity of all trajectories before and after stroke (one-way ANOVA, $F(1.6,6.3) = 1.94$, $p > 0.05$). Asterisks show Dunnett's multiple comparisons between the pre-stroke day and all post-stroke days of $p < 0.05$. Mean ± SD and single animals ($n = 5$).

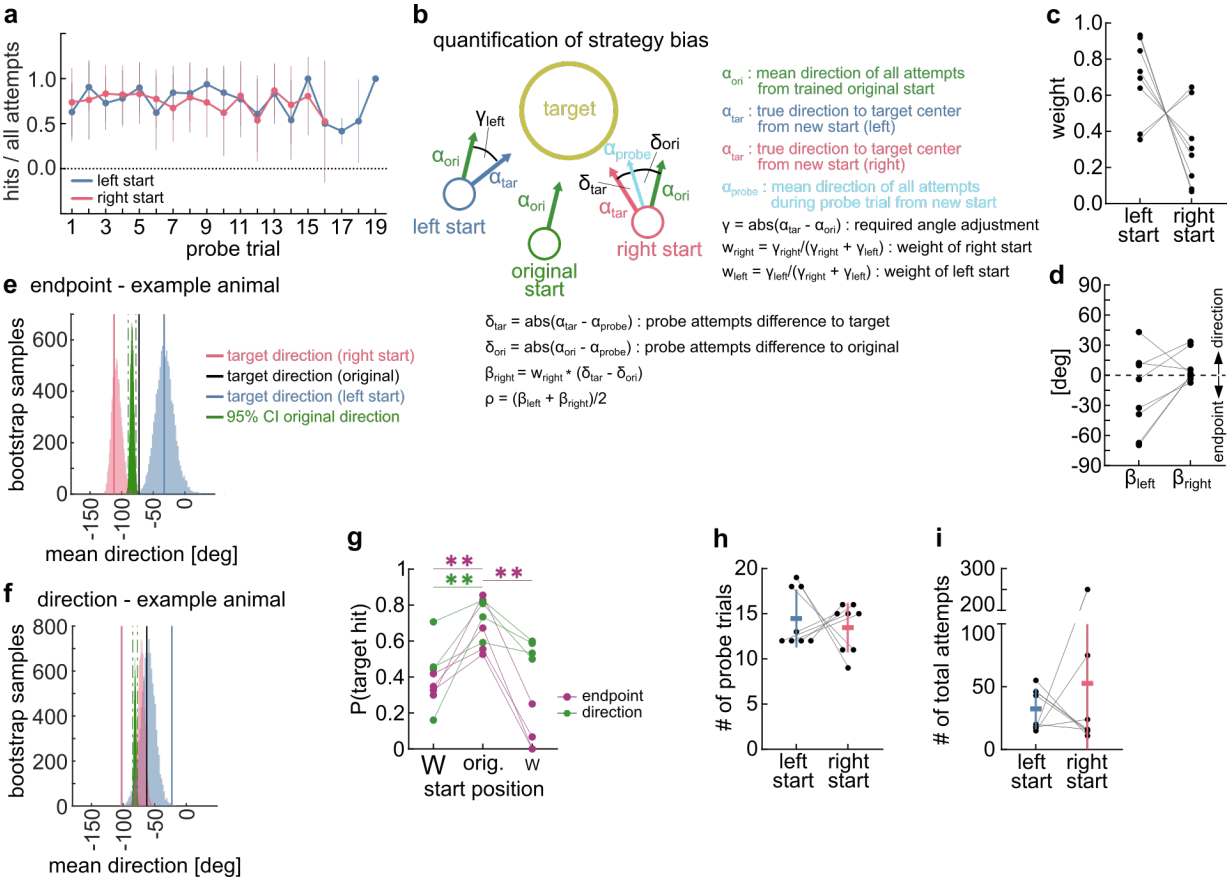

Figure S5: Quantification of endpoint-direction bias and fictive hit probabilities, Related to Figure 5.

(A) Hit ratio across probe trials (mean ± SD) for the left and right probe start positions showing there is no learning within the probe test session. **(B)** Schematic of the initial vector analysis used to calculate the weighted angles (β) and final angle (ρ) to quantify each animal's 'direction' or 'endpoint' learner bias (see STAR Methods for details). **(C)** Weighting factors for left and right probe starts determined from the difference angle (γ) between the original and target directions (α). **(D)** Weighted angels (β) for both probe start positions. **(E)** Mean initial direction distributions from bootstrapping for an example animal with an endpoint learner bias. **(F)** Same as (E) for example animal with direction learner bias. **(G)** Probability of target hit for attempts made from probe starts translated to original start positions compared to the true hit ratio in from the original start position. Start positions split by size of weight (w). **(H)** Total number of probe trials where the joystick moved to the left or right start for each animal (paired t-test: $t(7) = 0.48$, $p > 0.05$). **(I)** Total number of attempts performed from the probe start positions during all probe trials per animal (paired t-test: $t(7) = 0.62$, $p > 0.05$). Mean ± SD and single animals. $^{**}p < 0.01$.

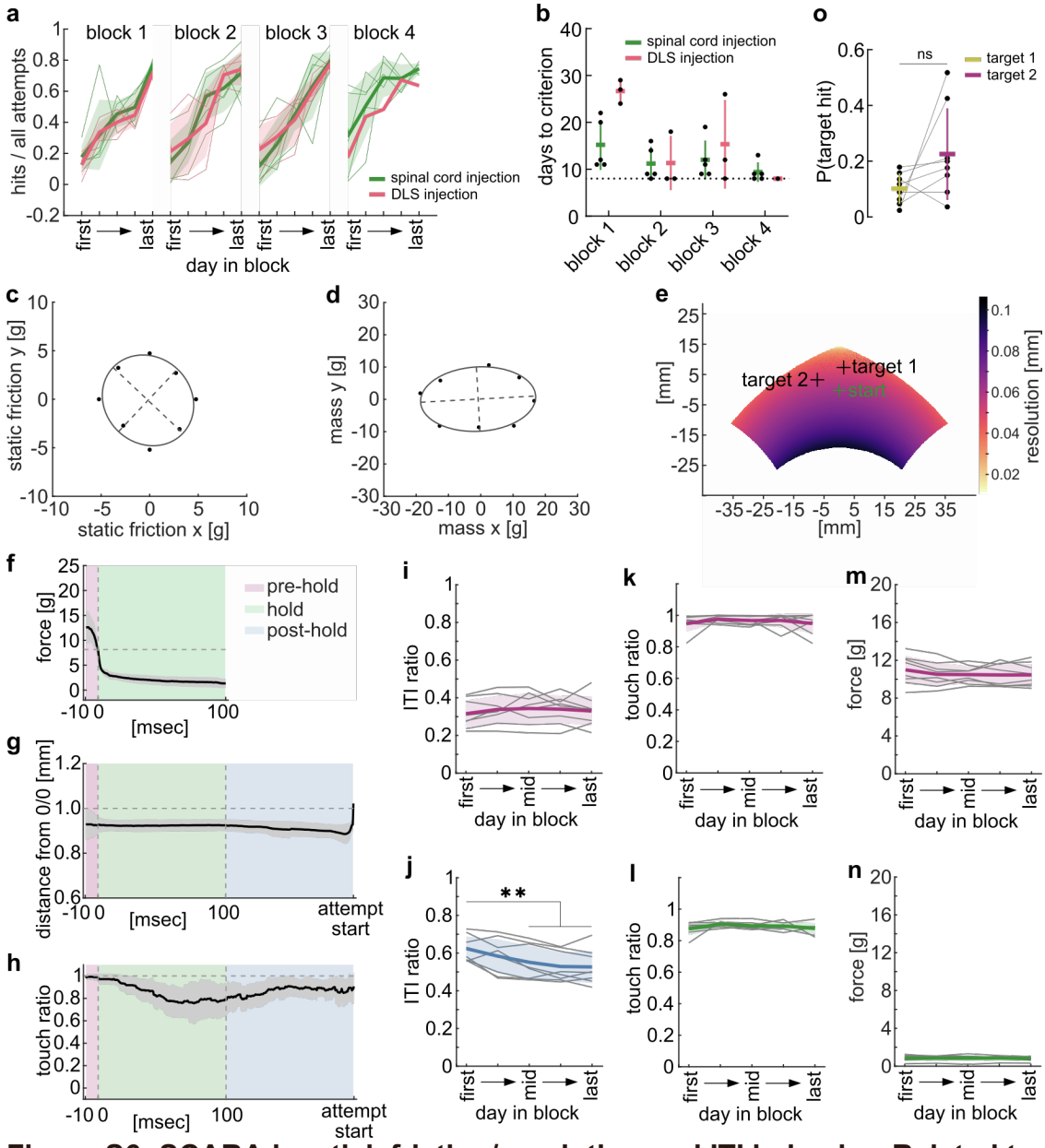

Figure S6: SCARA joystick friction/resolution and ITI behavior, Related to STAR Methods. **(A)** Hit ratio across all blocks of animals grouped by injection into the spinal cord or dorsolateral striatum (DLS) shows no difference in performance (Mixed-effects model, day: $F(3.8,20.9) = 12.34$, $p < 0.01$, group: $F(1,6) = 0.72$, $p > 0.05$, spinal cord injection: n = 5, DLS injection: n = 3). **(B)** Number of days in each block to reach the performance criterion shows no overall effect of injection location (Mixed-effects model, block: $F(3,16) = 7.68$, $p < 0.01$, group: $F(1,6) = 2.71$, $p > 0.05$, spinal cord injection: n = 5, DLS injection: n = 3). **(C)** Static friction at start position in 8 radial directions for an example setup. Average friction across all directions = 4.6 ± 0.7 g. **(D)** Mass of end-effector in 8 radial directions for an example setup. Average mass across all directions = 13.8 ± 3.2 g. **(E)** Workspace and spatial resolution of SCARA joystick with average target locations. **(F)** Force profile across all sessions of an example animal during the pre-hold and hold periods of the ITI. Horizontal dashed line shows the threshold of 7g. The force had to be below this threshold for 100 msec to exit the hold period. **(G)** Same example data as in (F) showing the joystick position during the pre-hold, hold, and post-hold periods. The post-hold period is resampled for all trials between the end of the hold period and the time point of leaving the start position (end of the ITI). The horizontal dashed line shows the radius of the start position. Crossing the radius initiates an attempt. **(H)** Same example data as (F/G)

showing the probability of joystick touch during the pre-hold, hold, and post-hold periods. **(I)** Ratio of ITIs that included a pre-hold period during which the animal exerted force above the threshold for at least 10 msec (one-way ANOVA rep. meas., $F(2.6,18.0) = 0.73$, $p > 0.05$). **(J)** Ratio of ITIs that included a post-hold period during which the animal was not yet leaving the start position after having exited the hold period (one-way ANOVA rep. meas., $F(2.7,18.8) = 13.18$, $p < 0.01$). **(K)** Probability of joystick touch during the pre-hold period (one-way ANOVA rep. meas., $F(2.8,19.5) = 0.82$, $p > 0.05$). **(L)** Probability of joystick touch during the hold period (one-way ANOVA rep. meas., $F(1.4,10.1) = 1.89$, $p > 0.05$). **(M)** Force exerted by the animal against the joystick during the pre-hold period (one-way ANOVA rep. meas., $F(1.9,13.3) = 1.42$, $p > 0.05$). **(N)** Force exerted by the animal against the joystick during the hold period (one-way ANOVA rep. meas., $F(2.1,14.4) = 0.56$, $p > 0.05$). **(I-N)** Analysis using all inter-trial-intervals (ITI) on 5 selected days and averaged across all blocks (n = 8 animals). One-way ANOVA with repeated measures, asterisks show Dunnett's multiple comparisons between the first day and all other days, $^{**}p < 0.01$. Mean ± SD and single animals. **(O)** Probability of entering defined targets with attempts made on the last day of pre-training (paired t-test: $t(7) = 1.76$, $p > 0.05$). **(A/B/O)** Mean ± SD and single animals.

Experiment	Animal ID	Trained in	Number of blocks	Test after block	AAV injection site	AAV	Provider / Lot number	Volume	Segments	Cortex lesion
Cup and Tube	T1222	Cup	1		No injection					
Cup and Tube	T1223	Cup	1		No injection					
Cup and Tube	T1224	Tube	1		No injection					
Cup and Tube	T1225	Tube	1		No injection					
Cup and Tube	T1226	Tube	1		No injection					
Cup and Tube	T1227	Cup	1		No injection					
Cup and Tube	T1228	Cup	1		No injection					
Cup and Tube	T1229	Cup	1		No injection					
Cup and Tube	T1230	Tube	1		No injection					
Cup and Tube	T1231	Tube	1		No injection					
Target Training	T222	Cup	4	5	Spinal cord	AAVretro(SL1)_Syn_GCamp6f	Janelia	75 nl/seg.	C4 – C8	No
Target Training	T223	Cup	4	5	Spinal cord	AAVretro(SL1)_Syn_GCamp6f	Janelia	75 nl/seg.	C4 – C8	Yes
Target Training	T224	Cup	4	5	Spinal cord	AAVretro(SL1)_Syn_GCamp6f	Janelia	75 nl/seg.	C4 – C7	Yes
Target Training	T225	Cup	4	5	Spinal cord	AAVretro(SL1)_Syn_GCamp6f	Janelia	75 nl/seg.	C4 – C7	Yes
Target Training	T228	Cup	4	5	DLS	AAVretro(SL1)_Syn_GCamp6f	Janelia	100 nl		Yes
Target Training	T229	Cup	3	3	DLS	AAVretro(SL1)_Syn_GCamp6f	Janelia	100 nl		No
Target Training	T230	Cup	3	3	DLS	AAVretro(SL1)_Syn_GCamp6f	Janelia	100 nl		Yes
Target Training	T635	Cup	4	3	Spinal cord	AAVretro_Syn_GCamp7f	21720	75 nl/seg.	C4 – C8	No
Whisker trim	T638	Cup	3		Spinal cord	AAVretro_Syn_GCamp7f	v52598	60 nl/seg.	C4 – C8	
Whisker trim	T640	Cup	3		DLS	AAVretro_Syn_GCamp7f	21720	80 nl		
Whisker trim	T644	Cup	3		DLS	AAVretro_Syn_GCamp7f	v52598	80 nl		

Table S1: List of all animals used in this study and their experimental procedures, Related to STAR Methods.

Figure	Panel	Mixed-effects model day in block effect (F)	Mixed-effects model day in block effect (p-value)	Mixed-effects model block effect (F)	Mixed-effects model block effect (p-value)
2	b	F(2.8,19.8) = 14.33	< 0.0001	F(1.7,12.0) = 3.24	0.082
2	c	F(2.4,16.9) = 7.68	0.003	F(2.4,16.8) = 0.73	0.521
2	g	F(2.1,14.8) = 8.46	0.003	F(2.1,14.5) = 0.91	0.428
2	i	F(2.2,15.3) = 15.31	0.0002	F(2.1,15.0) = 0.72	0.513
2	j (top row)	F(3.1,21.4) = 4.17	0.018	F(1.9,13.1) = 1.55	0.249
2	k / j (diagonal)	F(2.9,20.1) = 13.93	< 0.0001	F(2.1,14.4) = 0.16	0.862
2	l	F(1.9,13.4) = 5.22	0.022	F(2.3,15.9) = 1.43	0.269
Suppl. 2	e	F(2.4,17.0) = 0.93	0.431	F(2.0,13.7) = 2.08	0.163
Suppl. 2	f	F(1.9,13.0) = 9.60	0.003	F(2.4,16.9) = 0.97	0.414
3	b	F(1.4,9.7) = 7.54	0.016	F(1.6,11.1) = 2.35	0.147
3	c	F(2.4,16.8) = 11.43	0.0005	F(1.9,13.1) = 0.22	0.790
3	e	F(2.3,16.2) = 6.28	0.008	F(1.9,13.1) = 1.65	0.229
3	f	F(1.9,13.5) = 1.33	0.296	F(1.9,13.5) = 0.30	0.736
3	g	F(3.1,21.4) = 4.46	0.014	F(2.5,17.5) = 0.48	0.667
3	i	F(2.1,14.7) = 9.88	0.002	F(2.3,16.4) = 1.76	0.200
3	j	F(2.1,14.6) = 4.68	0.026	F(2.1,14.8) = 1.82	0.195

Table S2: Results from mixed-effects models on data before averaging selected days across blocks showing no significant effect of the block, Related to Figure 2/3.