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Supplemental Information

Directional Reaching for Water as a Cortex-Dependent Behavioral Framework for Mice

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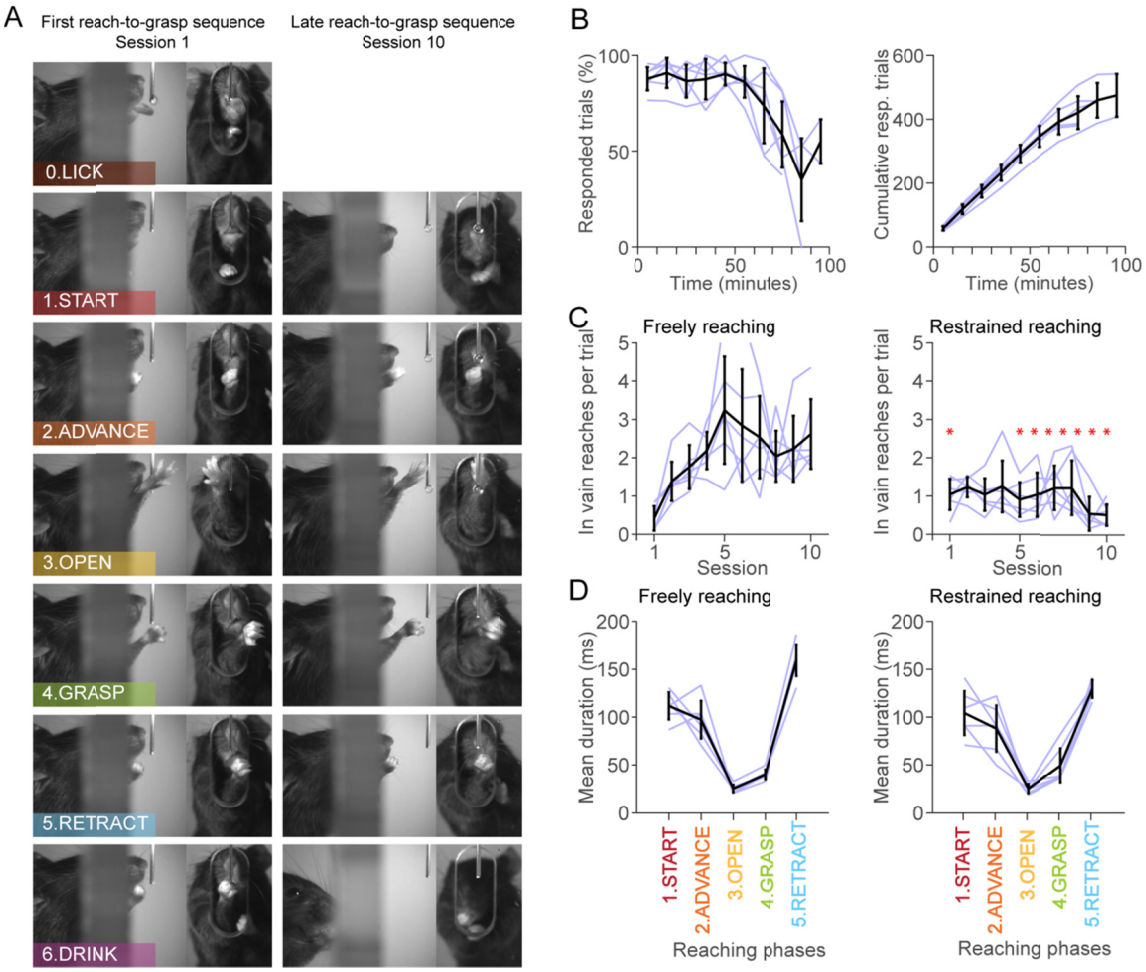

Figure S1. Reaching for water characterization. Related to Figure 1. (A) Example of the first reach-to-grasp sequence (left column) performed by a freely moving mouse after consuming the water through licking (LICK) which resembles the sequence of the same mouse after extensive training (session 10, right column). Note the position of the water spout in session 1 was closer to the box than in session 10. (B) Proficient mice were tested in a freely reaching session without time restriction (see Methods) to evaluate the engagement in the task and the maximal number of reaches performed. On the left, percentage of responded trials shows that mice were engaged in reaching for at least 60 minutes. On the right, the cumulative number of responded trials shows that mice performed a total of 438 ± 60 trials. Data binned in 10 minute intervals (C) Freely moving mice tend to perform more "in-vain" reaches (see Methods) per trial than head-fixed mice in later sessions (* $p < 0.01$ Tukey post hoc test after significant session per treatment interaction RM ANOVA). (A-C) Black lines mean ± s.d.; light lines individual animals. (D) Mean duration of the different phases of the reach-to-grasp sequence are comparable between freely moving and head-fixed mice (see table 1). (B-D) Black lines are the mean ± s.d.; thin lines individual animals.

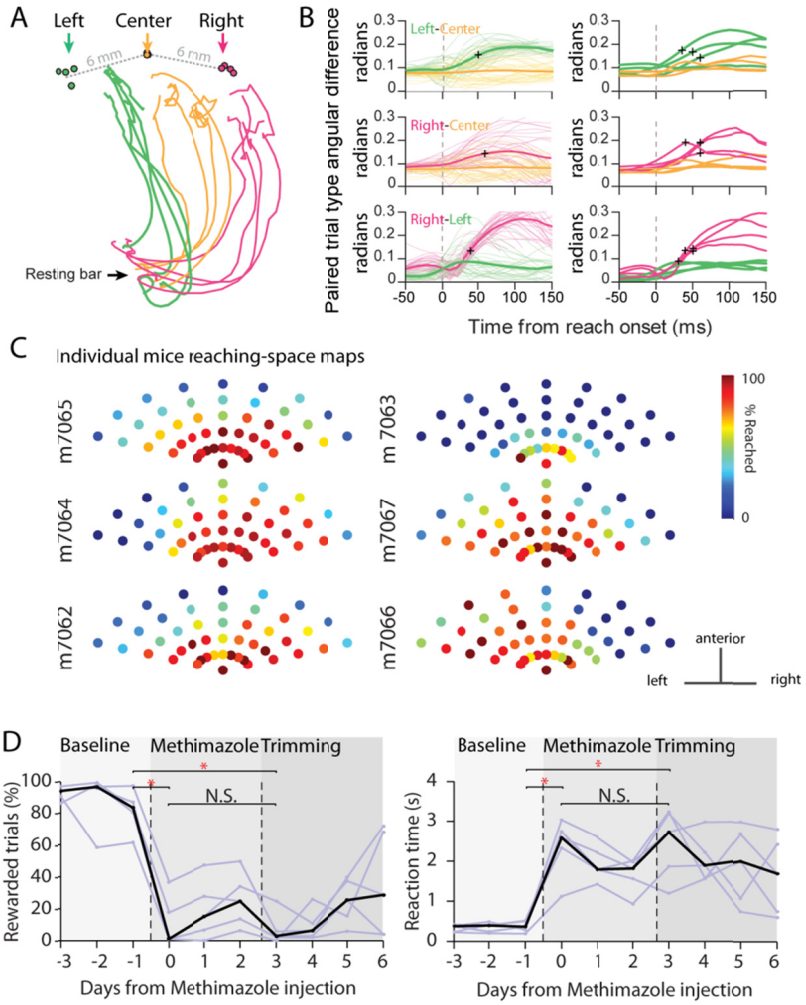

Figure S2. Multiple-target reaching and sensory guidance characterization. Related to Figure 2. (A) Top view of average reaching trajectories for N = 4 mice to the left, center and right targets. (B) Pair-wise angular difference between two trial types. Top: Left trials against the average of central trials (left-central, green) and central trials against the average of central trials (central-central, yellow). Middle: Right-central (magenta) and central-central (yellow). Bottom: Right-left (magenta) and left-left (green). Black crosses indicate statistically significant ($p < 0.001$, Kuipers circular test) differences between the angular differences of the corresponding trial types compared. Left column: one session for one example animal; thin traces individual trials, thick traces average. Right column: data from 4 mice (average angular difference within a session). Mean divergence time: 49.1 ± 10 ms from reaching onset. (C) Individual reaching space maps. (D) Performance (left) and reaction time (right) across consecutive sessions to study the effect of pharmacological lesion (methimazole i.p. injection) of the olfactory epithelium (N=5 mice). After methimazole injection performance significantly dropped and reaction times increased. Subsequent testing showed a recovery trend (although statistically not significant) which was subsequently abolished by whisker trimming. * p <0.001 (rewarded trials) and * p = 0.002 (reaction times), Tukey post hoc test, RM ANOVA. Black lines median across animals; light lines individual animals.

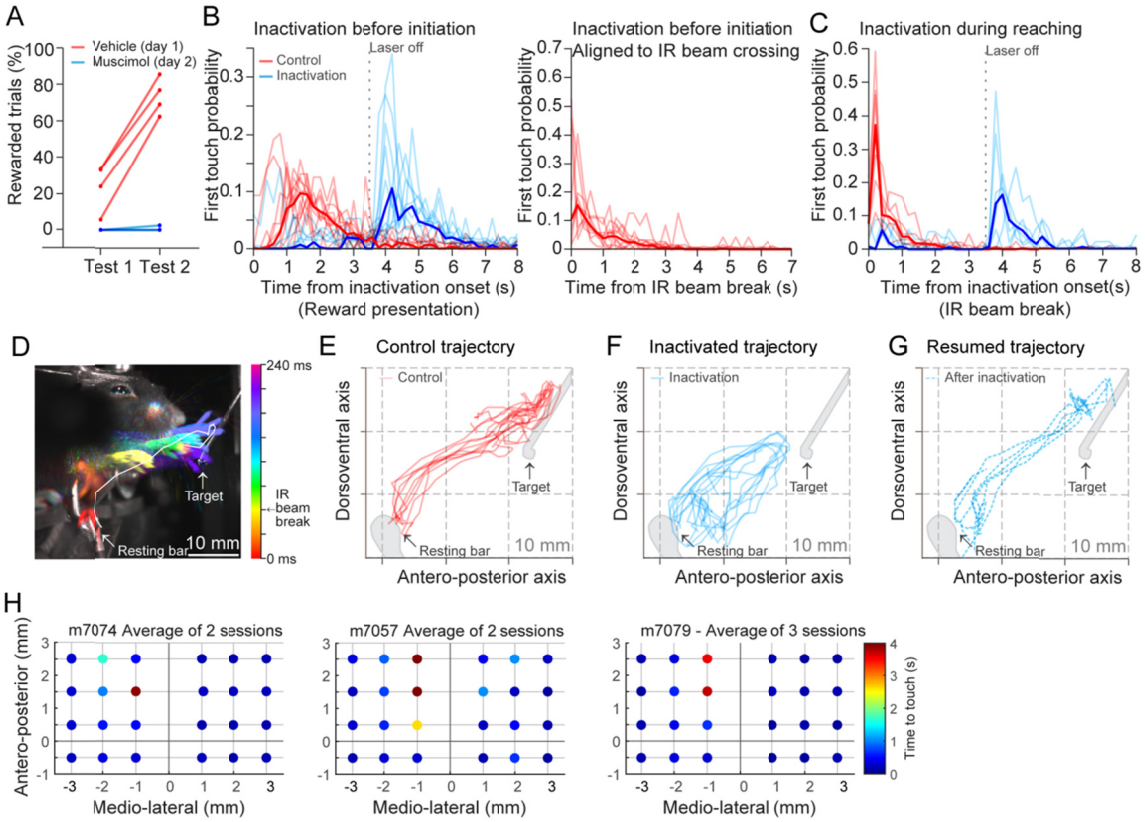

Figure S3. Cortical inactivation characterization. Related to figure 3. (A) 75 nl muscimol (5 mg/ml) injection in the motor cortex precludes mice from performing goal directed reaches under head-fixation. (B) On the left, touch probability of the water spout as a function of time for control (red) and inactivated trials (blue). Laser illumination was turned on simultaneously with water drop presentation (i.e. inactivation before initiation of reaching). During inactivated trials, affected mice (N = 10) initiated reaching after laser illumination was turned off. However, some mice occasionally overcame the optogenetic inactivation effect and managed to perform reaching movements and touch the water spout during the inactivation period. Right panel, the same data as on the left panel for control trials aligned to IR beam break for comparison with control data in C. (C) Touch probability plot for inactivation experiments during ongoing reaching. In inactivated trials, laser illumination was turned on at the time of IR beam crossing. Affected mice (N = 6) reached the water spout after the end of laser illumination. In some trials, mice overcame the inactivation effect and reached the target while laser illumination was on. (D) Lateral view colored projection of selected frames of video recorded data highlighting the position of the paw during the reaching trajectory of a control trial. Time references in the color bar on the right. The manually reconstructed trajectory is overlaid in white. (E-F) Reconstructed reaching trajectories of a representative mouse under control (E) and inactivated (F-G) trials. Trajectories in G correspond to the reaching movements performed at the end of the inactivation period of inactivated trials. (H) Individual inactivation maps.

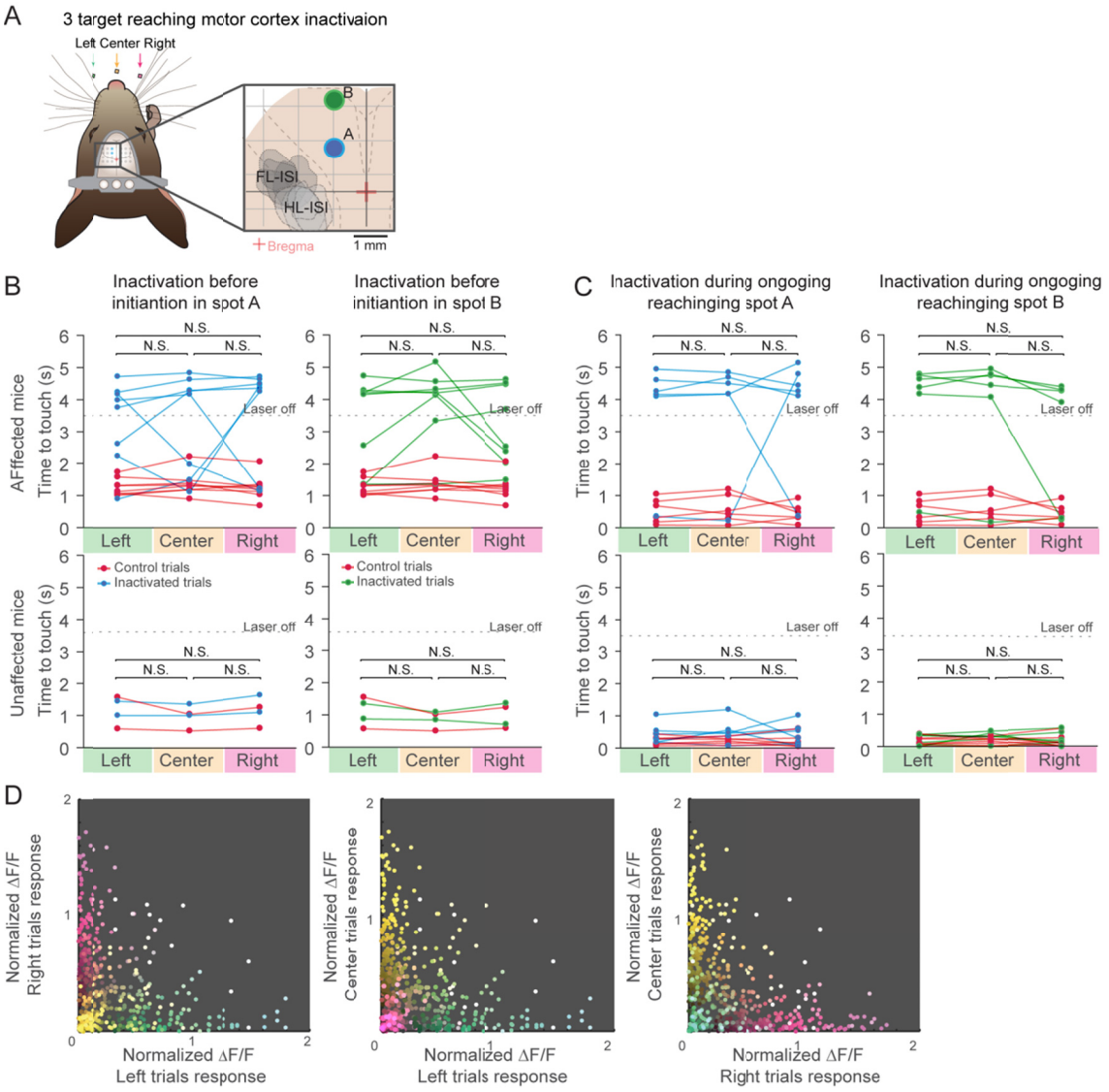

Figure S4. Involvement of secondary motor cortex in directional reaching. Related to figure 4. (A) Schematics of spot A and spot B optogenetic inactivation during the directional reaching task. Red cross: bregma; dotted lines: borders of main cortical regions according to Allen Brain Atlas. Gray shades correspond to the intrinsic signal of forelimb (FL-ISI) and hindlimb (HL-ISI) of the same mice used in the inactivation experiments. (B) Optogenetic inactivation before reaching initiation in spot A (left column) or B (right column) produced similar behavioral effects irrespective of trial type (left, center or right) in mice affected by optogenetic inactivation (top row). On average, unaffected mice did not show higher probability of being affected for any of the trial types (bottom row). (C) Same as (B) but inactivation during ongoing inactivation. (D) Same data as Figure 4C projected into a 2 dimensional space.

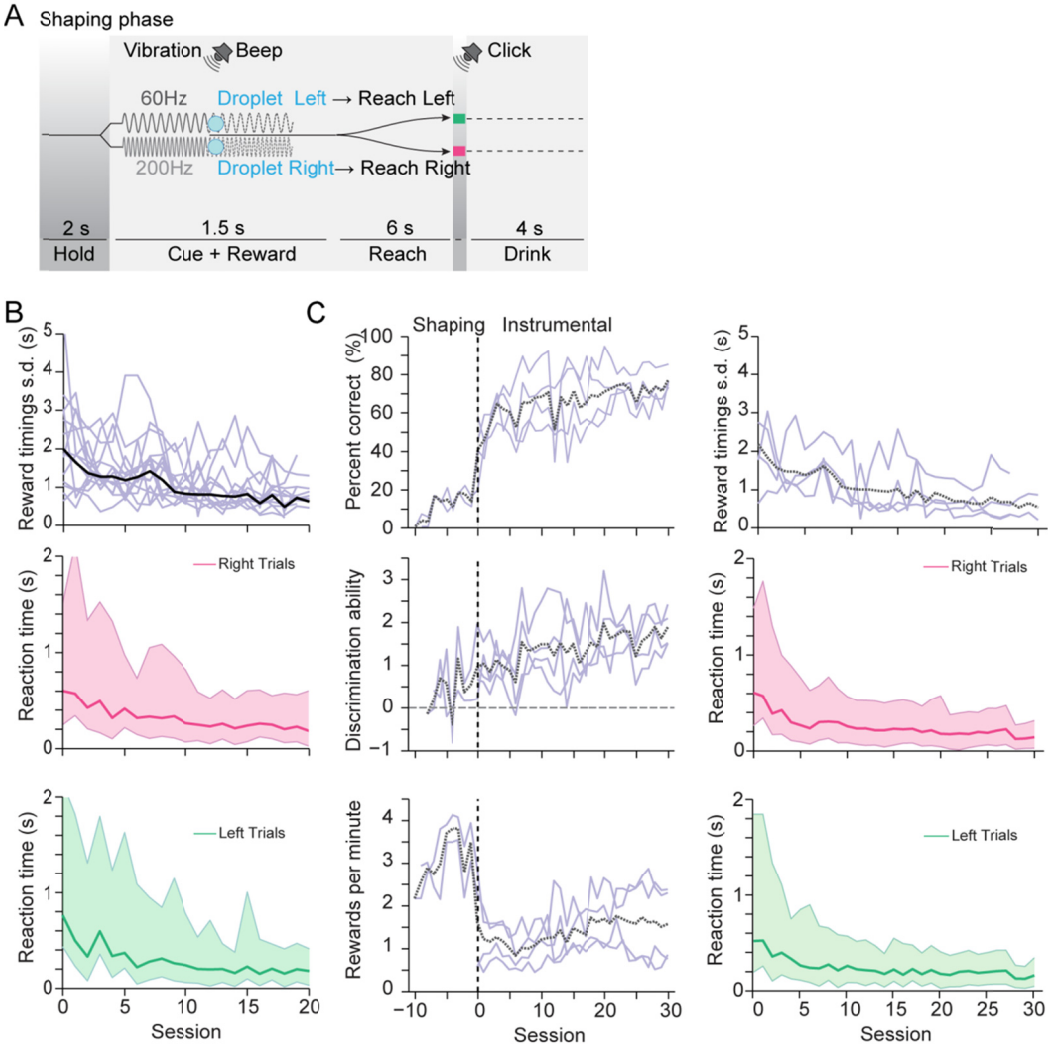

Figure S5. Training of instructed reaching task. Related to Figure 5. (A) Schematics of the training structure during the handling period (shaping phase) showing the sequence of events in a trial. During this phase, mice were not forced to perform instrumental reaches in order to get a reward. After 0.6-1 s of the beginning of the vibratory cue the "go" cue was played and the reward was delivered (60 Hz → left target, 200 Hz → right target) to induce reaching for water movements to the correct target. Mice were then allowed to reach during a time window of 6 s before a new trial started. If mice reached to the correct target within this time, a "click" sound was played and mice were allowed 4 s to drink. As training progressed, the reward delivery was gradually delayed respect to the "go" cue. Under these circumstances mice were able to perform anticipated reaches (i.e. reaching before the droplet was delivered). When anticipated reaches surpassed ~20-30 % of the trials, the shaping phase of the training was finished. (B) Reward timings dispersion and reaction time across sessions of mice depicted in Figure 5G-I to show the distribution of the data. Black trace in reward timings is the mean of 14 mice (light traces individual animals). Pink and green solid lines are the median of the population reaction time; shaded areas cover the 10-90 % quantiles of the distribution. (C) Learning curves of a subset of mice that were trained more extensively than 20 sessions showing not major improvements in behavior.