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Supplemental Information

A Cortical Substrate for Memory-Guided Orienting in the Rat

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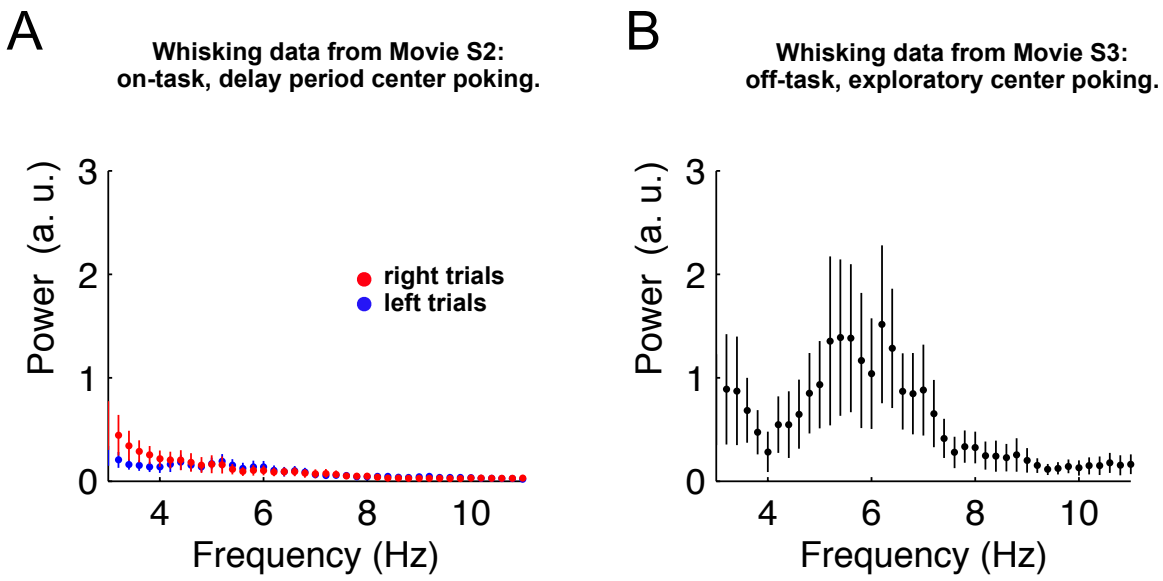


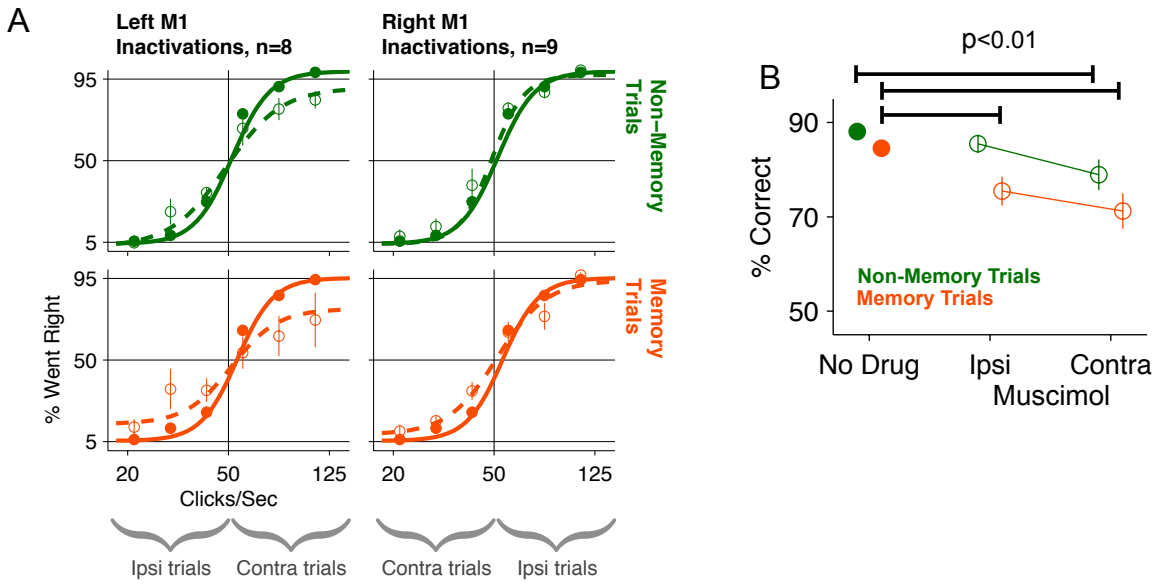
Figure S1, No significant difference in whisking between right choice and left choice trials when the animals are performing the task. Related to Figure 1.
Quantification of the whisking data collected in **Movie S2 and S3**; see caption to those videos for an explanation of the source and form of the whisking data. The initiation time of each trial in our memory-guided orienting task is controlled by the subject placing its nose in the center port. Rats do not initiate trials at consistently identical intervals, but instead perform the task in bouts, typically performing many tens of trials in quick regular succession (we call these time periods “on-task”) followed by short breaks (typically, a few minutes) of grooming and sniffing/whisking as they move around the behavior box (“off-task”). (**A**) Data from **Movie S2:** minimal whisker motion, and no significant whisking difference between left trials and right trials during on-task center pokes. The panel shows a spectral analysis of whisking during the “nose in center” delay period from randomly selected, correctly completed left and right memory trials. (**B**) As in panel A, but from center pokes during off-task periods (**Movie S3**). The clear peak at the theta frequency (~ 6 Hz) during these off-task center pokes demonstrates that animals are capable of producing measurable whisking motions while their snouts are in the center poke. Data in panels (A) and (B) suggest that during on-task center poking, rats choose to not whisk.

Movie S1. Strong, clearly visible whisking during exploration of the behavior box at the beginning of a session.
To facilitate visualizing the whiskers, we painted several dots of titanium dioxide onto them, as well as affixing a small piece of aluminum foil to one of the right whiskers. At the start of each session, before performing any trials, rats typically sniff and whisk briskly around the behavior box. The video shows a rat during this initial exploration period, before performing any trials in the session. The video demonstrates that the titanium dioxide and aluminum foil do not affect the rat's ability to rapidly whisk as it performs its exploratory motions.

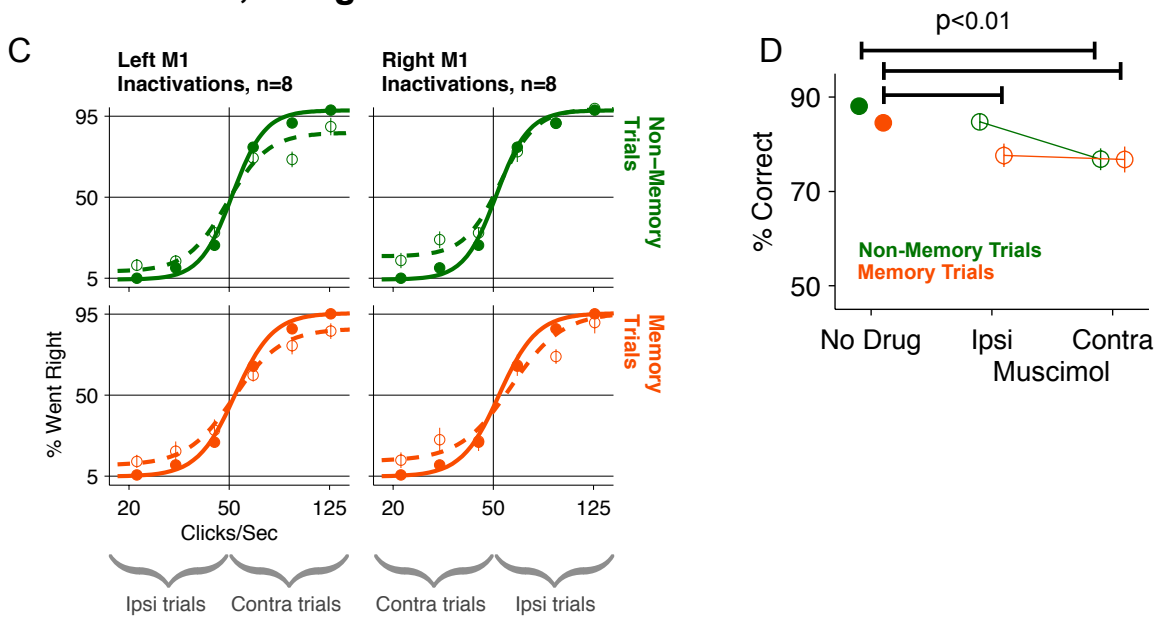
Movie S2. Minimal whisking during on-task center poking
The video shows that when rats are performing the task, whiskers are held very still during delay period center poking. Data from this video is quantified in **Figure S1A.** Correct left or right memory trials were randomly selected for whisker position scoring. Trials were included if the rat's head remained in a relatively horizontal position for the duration of the trial, so that whiskers were visible throughout the trial and their position could be scored. Scoring was performed blind as to whether a trial was a right-instructed or a left-instructed trial. The video is 5x slower than real time to facilitate observation of the whiskers during the delay period. The rat in this video was cannulated, and the cannula sites were used as fiducial markers on the head. Two points on a right whisker were marked in every frame as fiducial points on the whisker. The distance from the distal whisker point to the right head fiducial point is marked with a red line. This distance (*l,* length of the red line) was used as a measure of whisker position. A spectral analysis of *l(t)* is shown in **Figure S1A**.

Movie S3. Visible whisking during off-task center poking
The video shows that rats are capable of whisking even when their snouts are in the center port. Data from this video is quantified in **Figure S1B.** During off-task pauses between runs of completed trials, rats will sometimes sniff and whisk around the behavior box, including occasionally nose poking into the center port. The video shows selected bouts of center poking that were defined as "off-task" in that no completed trials occurred closer than 5 seconds, neither before nor after the time period shown in the video. For example, in one of these periods the rat center poked during the inter-trial interval. In another, the rat initiated a regular center poke during the "nose-in-center" period, but then did not complete it and did not return to complete a trial until 5 seconds later. Scoring methods and conventions as in **Movie S2.**

M1, 0.5 mg/ml muscimol = Equal to the dose in FOF



M1, 1 mg/ml muscimol = Double the dose in FOF



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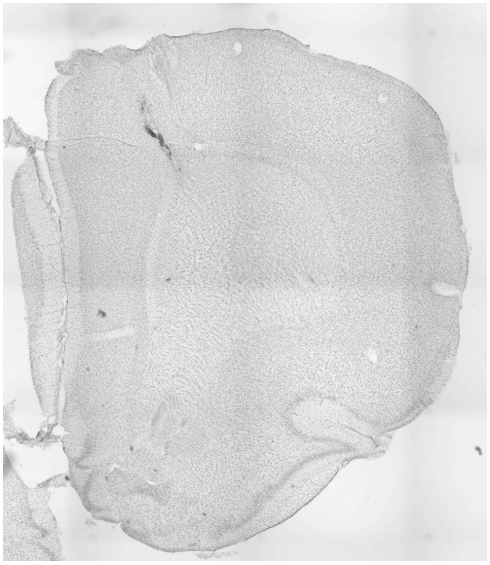
E

Example FOF histology

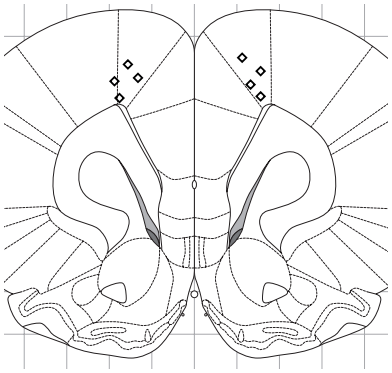


F

Example M1 histology

G

FOF histology



AP=2.52 from Bregma

H

M1 histology

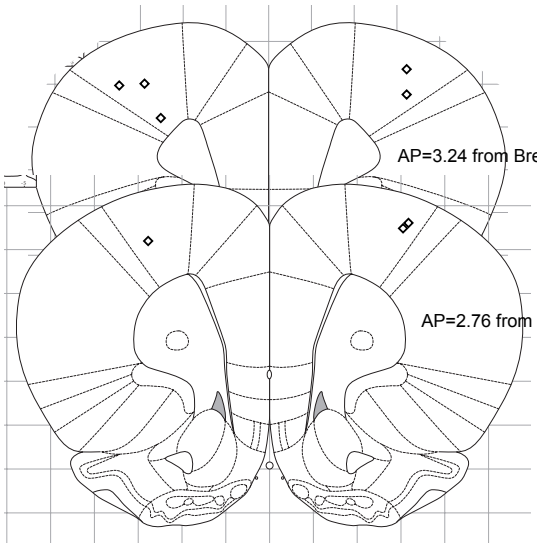


Figure S2, Unilateral inactivation of neck motor cortex (M1) produces a different and much weaker pattern of impairment than inactivation of the FOF. Related to Figure 2.

(**A**,**C**) Behavioral performance in control and muscimol-infusion days for 0.5 mg/ml (**A**) and 1 mg/ml (**C**) muscimol. Top row: non-memory trials. Bottom row: memory trials. Left column: muscimol infusions into left M1. Right column: muscimol infusions into right M1. Open circles, data from muscimol infusions. Closed circles: control data from days immediately preceding infusion days. Dashed lines: sigmoidal fits to muscimol data. Solid Lines: sigmoidal fits to control data. Error bars are standard error of the mean across sessions (The number of sessions is indicated at the top of each plot). Error bars for control data were smaller than the marker in most cases. Underbraces at bottom indicate the sets of trials in which animals were instructed to orient ipsilaterally or contralaterally to the site of infusion. (**B**,**D**) Combined data from left and right infusion sessions at 0.5 mg/ml (**B**) and 1mg/ml (**D**) of muscimol and collapsed across all stimulus difficulty levels. The "No Drug" data come from the 20 sessions one day before infusion sessions. The Ipsi and Contra Muscimol data are the performance on ipsilateral trials and contralateral trials on infusion sessions (n=20). **(E)** Example of a nissl stained coronal section from a rat with cannula implanted in the FOF. The section is ~2.6 mm anterior to Bregma. **(F)** Example of an unstained bright field coronal section from a rat with cannula implanted in M1. The section is ~3.3 mm anterior to Bregma. (**G**) FOF cannula placements. Diamonds indicate the location of the scar left from the injection cannula for each rat. **(H)** M1 cannula placements. Diamonds indicate the location of the scar left from the injection cannula for each rat.

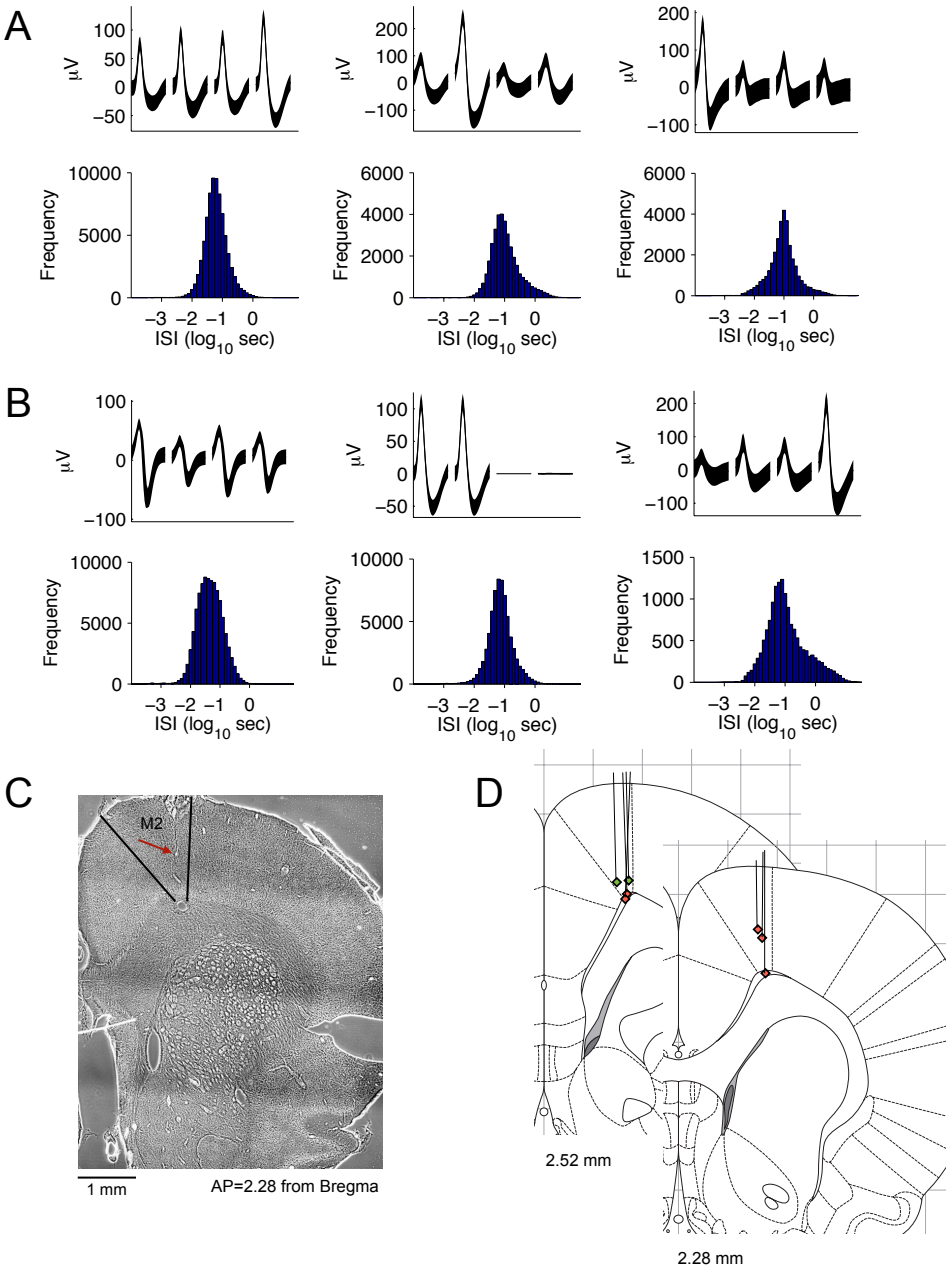


Figure S3, Waveforms and Inter-Spike Interval histograms for example cells in figure 3, and histological placement of electrodes. Related to Figure 3.
(A) Waveforms (mean ± standard deviation) and inter-spike interval histograms for the three contralateral preferring neurons shown in **Figure 3A**. (**B**) Waveforms (mean ± standard deviation) and inter-spike interval histograms of the three ipsilateral preferring neurons shown in **Figure 3B**. (**C**) Example of an electrode track in FOF. The thin black lines indicate the borders of M2 (Paxinos and Watson, 2004). (**D**) Electrode tracks for the 5 implanted rats. **Green** diamonds indicate the final location of the tips from electrodes in the **left FOF** and **red** diamonds indicate the final location of the tips from electrodes in the **right FOF**.

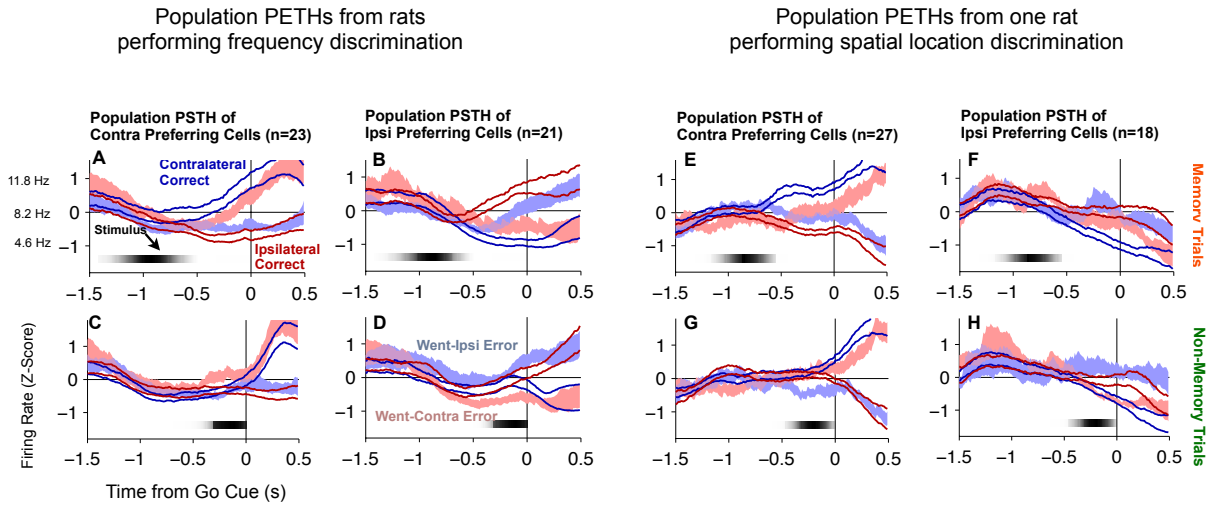


Figure S4, Comparison of electrophysiological results for frequency discrimination vs spatial location discrimination. Related to Figure 4.

(**A-D**) **Population PETHs for rats performing frequency discrimination** (n=44). Each panel is a population perievent time histogram (PETH) showing the average response (across neurons) on correct (thick lines, mean±std.err. across neurons) and error trials (shaded, mean±std.err. across neurons) where the stimulus indicated that the correct response was contralateral (blue) or ipsilateral (red) to the recorded neuron. PETHs are aligned to the time of the Go cue (center LED offset). (**A**) The average responses of memory trials for neurons that fired more on contralateral trials. (**B**) The average responses of memory trials of neurons that fired more on ipsilateral trials. (**C**) Same as A but for non-memory trials. (**D**) Same as B but for non-memory trials. (**E-H**) **Population PETHs for rats performing spatial location discrimination** (n=45) Each panel is a population PETH showing the average response (across neurons) on correct (thick lines, mean±std.err. across neurons) and error trials (shaded, mean±std.err. across neurons) where the stimulus indicated that the correct response was contralateral (blue) or ipsilateral (red) to the recorded neuron. PETHs are aligned to the time of the Go cue (center LED offset). (**E**) The average responses of memory trials for neurons that fired more on contralateral trials. (**F**) The average responses of memory trials of neurons that fired more on ipsilateral trials. (**G**) Same as **E** but for non-memory trials. (**H**) Same as **F** but for non-memory trials.

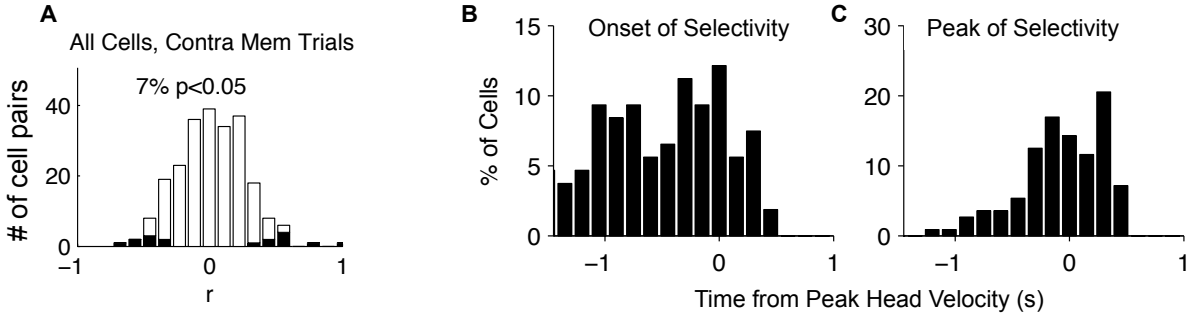
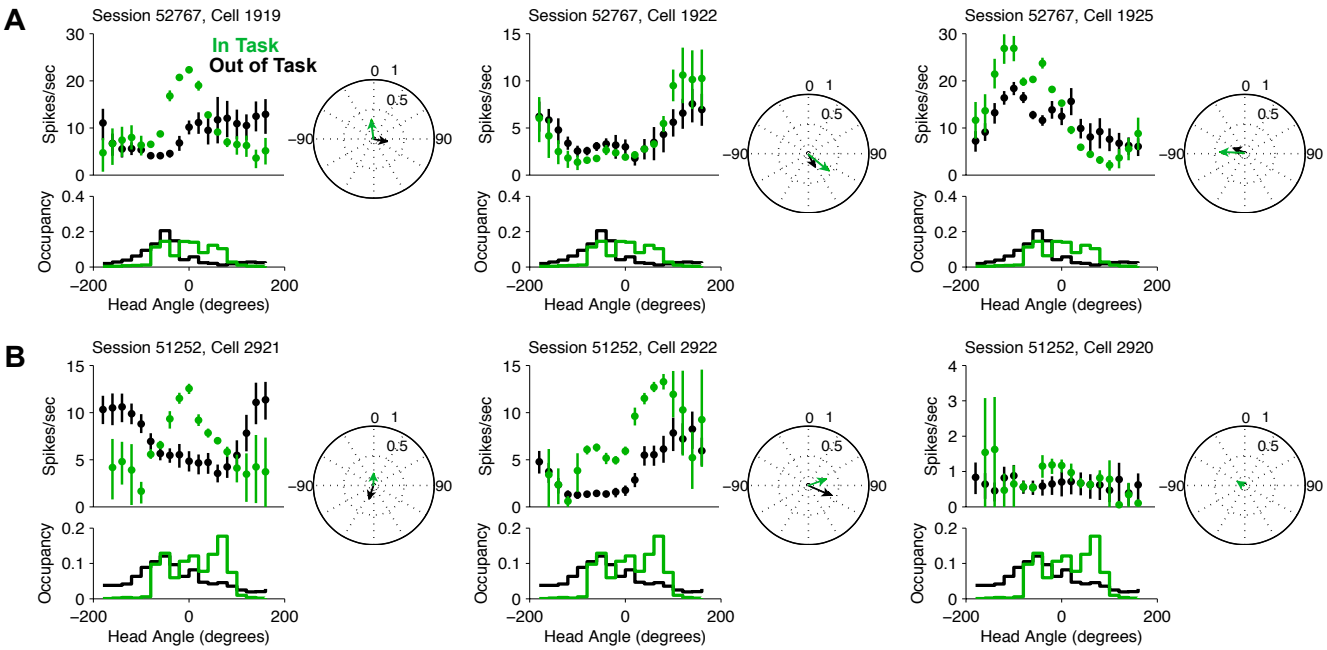
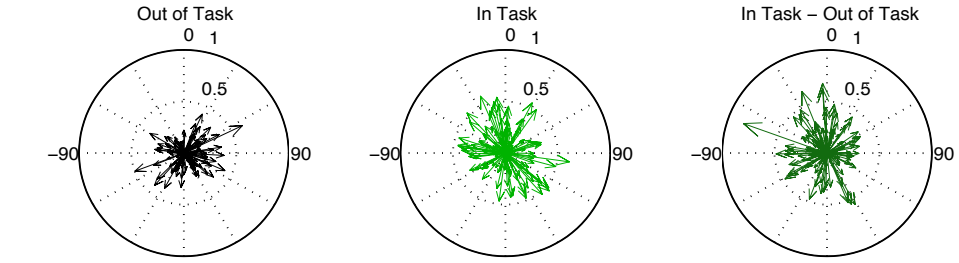


Figure S5. Neural latency is not correlated across pairs of simultaneously recorded neurons. Related to Figure 5.
(A) Histogram of the correlations in neural latency (as computed in Figure 5) for 238 pairs of neurons. The population is not significantly different than zero, nor is the number of individually significant neurons (7%) significantly more than expected at $p<0.05$. (**B**) Histogram of the time difference between the onset of differential ipsi vs. contra firing rate (as measured by a sliding ROC, as in **Figure 3A**,**B**) and the time of peak head velocity. Each entry in the histogram is a neuron; the histogram shows all 166 neurons recorded during sessions with head-tracking data. One hundred and forty-six (88%) of the neurons had a differential firing rate onset that preceded the time of peak head velocity. (**C**) As panel (B), but now showing the difference between the time of the largest differential ipsi vs contra firing rate and the time of peak head velocity. One hundred and twenty-four (75%) of the neurons had the peak of their differential firing rate occur before the time of peak head velocity.


A
Session 52767, Cell 1919
In Task
Out of Task
Spikes/sec
Occupancy
Head Angle (degrees)
Session 52767, Cell 1922
Session 52767, Cell 1925
B
Session 51252, Cell 2921
Session 51252, Cell 2922
Session 51252, Cell 2920


C
Out of Task
In Task
In Task – Out of Task

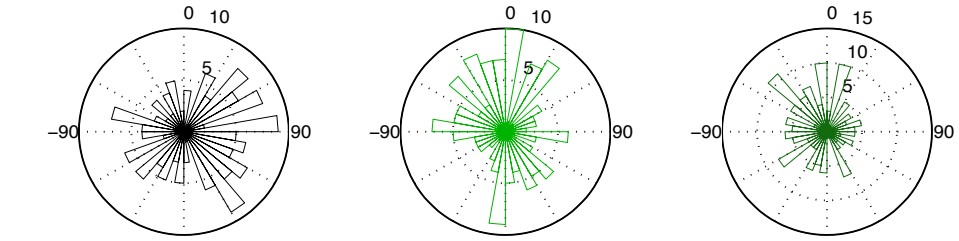

D

Figure S6. Head direction tuning in FOF. Related to Figure 6
(A) Head direction tuning and occupancy histograms for three cells recorded simultaneously in a single session. Tuning and occupancy were computed separately for in-task epochs (green; any timepoint within 10 seconds of a poke into any of the nose ports) and for out-of-task epochs (black; all other timepoints). Occupancy histograms indicate fraction of time spent with the head pointing in each direction. Error bars for firing rates are bootstrapped 99% confidence intervals of the firing rate at each of 18 bins of head angle. The center port is at 0 degrees. The compass plots to the right of each tuning histogram summarize the in-task and out-of-task tuning. Each cell's summary vector is the weighted average of a set of unit length vectors pointing in each of the 18 head direction bins; the weighting was proportional to the cell's firing rate in each direction bin. The summary vectors show how each cell contributes to the plots in panel C. **(B)** Same as A for three neurons recorded during a different session. **(C)** Summary of head-direction tuning for 166 cells recorded during sessions with head-tracking data. Each cell is represented by a summary vector, shown as a single arrow. The rightmost panel is the vector difference, for each cell, between its in-task summary vector minus its out-of-task summary vector. **(D)** Polar histogram of the angles of the arrows in **C**. The distribution of angles for In Task - Out of Task is significantly non-uniform (Omnibus Test, $p<0.03$)

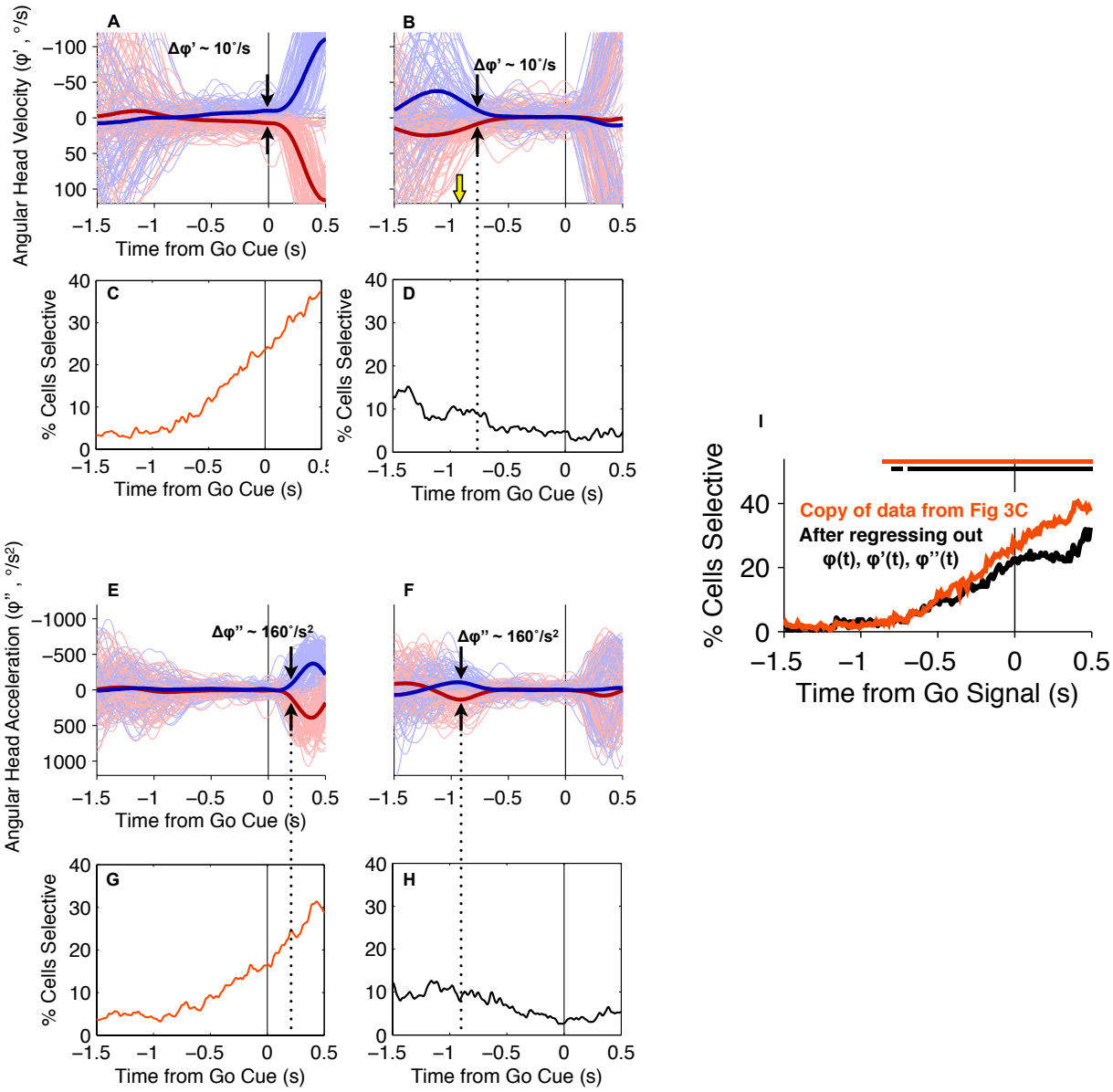


Figure S7. Predictive coding of response is not a simple function of current angular head velocity or acceleration. Related to Figure 7

(A) Angular head velocity (φ', deg/s) as a function of time relative to the Go cue for correct memory trials. Thin blue lines are from a random subsample of trials where the rat's final choice was to move left; thin red lines are a random subsample of trials where the final choice was to move right. Thick lines are the mean φ'(t) for each group, averaged over all correct memory trials. At the time of the Go cue (t=0), the difference in head velocity between the two groups is approximately 10 deg/s. **(B)** As in panel A, but with the grouping defined by the sign of the angular head velocity at time t=−0.9 sec (yellow arrow; φ'(t=−0.9)>0 versus φ'(t=−0.9)<0). At t=−0.78 sec, the difference in head velocity between the two groups is approximately 10 deg/s. **(C)** Percentage of cells (out of 166 neurons) with significantly different firing rates for the red vs blue groups of panel A. At each timepoint, threshold for each cell being considered significant was p<0.01. At t=0, 23% of cells have firing rates that discriminate between the two groups. (Continued on next page)

(D) As in panel C, but for the $\varphi'(-0.9)>0$ versus the $\varphi'(-0.9)<0$ groups. At t=−0.78 sec, the difference in head velocity between the two groups is approximately 10 deg/s, but only 9% of cells discriminate between the two groups.**(E, F, G, H)** As in panels A,B,C,D, but for angular head acceleration (φ'', deg/s^2). **(E)** 200 ms after the time of the Go cue (t=0.2), the difference in φ'' between the two groups is approximately 160 deg/s^2. **(F)** As in panel E, but with the grouping defined by the sign of φ'' at time t=−0.9 sec ($\varphi''(t=-0.9)>0$ versus $\varphi''(t=-0.9)<0$). At t=−0.9 sec, the difference in head velocity between the two groups is approximately 160 deg/s^2. **(G)** Percentage of cells (out of 166 neurons) with significantly different firing rates for the red vs blue groups of panel E. At each timepoint, threshold for each cell being considered significant was $p<0.01$. At t=0.2, 23% of cells have firing rates that discriminate between the two groups. **(H)** As in panel F, but for the $\varphi''(-0.9)>0$ versus the $\varphi''(-0.9)<0$ groups. At t=−0.9 sec, the difference in head acceleration between the two groups is approximately 160 deg/s^2, but only 9% of cells discriminate between the two groups. **(I)** Development of choice-dependent activity over the course of the trial after accounting for the effects of head angle variables. The orange line is copied from **Figure 3C.** The black line represents, at each timepoint, the percentage of cells (out of n=166) with significantly different ipsi vs contra residual firing rates. Residual firing rates are obtained after performing a linear regression to eliminate each cell's firing rate dependencies on $\varphi(t)$, $\varphi'(t)$, and $\varphi''(t)$ (see Supplementary Experimental Procedures). The black and orange horizontal lines at the top of the panel indicate timepoints for which the percentage of significant cells is more than expected by chance.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES

Subjects

Animal use procedures were approved by the Princeton University Institutional Animal Care and Use Committee and carried out in accordance with National Institutes of Health standards. All subjects were male Long-Evans rats (Taconic, NY). Rats were pair-housed during initial behavioral training and then single housed after being implanted with electrodes or cannula. Rats were kept on a reverse 12-hour light dark cycle and trained in their dark cycle, when they were more active. Rats were placed on a restricted water schedule to motivate them to work for water reward.

Behavior

Behavior took place in a custom training box (Island Motion, NY) inside a sound and light attenuating chamber (H10-24A, Coulbourn Instruments, PA). In each box there were three "nose ports", conical openings into which the rats could poke their snouts. The three nose ports were arranged side-by-side along a curved wall. Each nose port had an infra-red (IR) beam across the front (used to detect when the rat's nose was in the port), a visible white light emitting diode (LED), and a sipper tube connected to a water supply that was controlled by a computer controlled solenoid. In addition, there were two speakers mounted above each of the left and right nose pokes. All aspects of the task were computer controlled. Behavioral events were timestamped with greater than 1ms accuracy using a custom open-source software (Island Motion, NY; open-source code at http://code.google.com/p/rt-fsm/) on a computer running a realtime linux operating system. Rats were placed in and removed from the behavior box by technicians that were blind to the task.

Rats were trained using an automated training protocol. First, rats were trained using a classical conditioning paradigm. On each trial a sound played out of one of the speakers and then water was delivered from the corresponding port. The goal of this stage was simply to teach the rat that the ports delivered water that was contingent on sounds. The trials during this training stage had an inter-trial interval of about 2 minutes. The side of the sound and water delivery was alternated each day. This stage lasted 8 days. The next stage involved the rats learning to poke in the center port in order to initiate a trial. A light would come on in the center port and when the rat poked that would trigger the presentation of sound. The sound was a click train, and the rats had to learn an association between the rate of the clicks and the associated side for reward. For example, 100 clicks/sec meant reward was on the left and 25 clicks/sec meant the reward was on the right. At first, trials were presented in blocks of right and left trials. As the rat showed that he had learned the basic task structure we moved to randomly interleaved right and left trials (about 1 week).

The next stage of training was growing the 'fixation period'. At first, the rat could essentially just poke in the center and then respond in a side port. We incrementally grew the time that the rat had to stay in the center port before making his response. The end of the fixation period was indicated by extinguishing the center light. Once rats could maintain fixation for one second we moved to the next training stage. Rats were highly variable as to how long it took them to pass this stage (1-4 weeks). Until now the sound played for the entire duration of the trial. That is, there was no memory requirement. The next stage slowly shortened the duration of the stimulus to 300 ms. This stage took 2-5 weeks depending on the performance of the rat. At the end of this stage the rat was doing randomly interleaved, left and right memory trials. The next stage introduced no-memory trials where the stimulus was played at the end of the fixation period (**Figure 1A**). The final stage introduced intermediate stimuli in order to produce psychometric discrimination data. Rats learned that click trains < 50 clicks/sec meant reward on the right and > 50 clicks/sec meant reward on the left. In each session 6 discrete sounds were played (E.g. 100, 80, 60, 40, 30, 20 clicks/sec) and the values of the sounds were adjusted at the end of each session to better sample the psychometric curve. So, a rat with very good discrimination might end up with 100, 60, 52, 48, 40, 20 clicks/sec as the set of stimuli. The rats in the M1 muscimol experiment learned the opposite rule: click trains > 50 clicks/sec meant reward on the right and < 50 clicks/sec meant reward on the left.

All data described in this paper was collected from rats in the final training stage. Sessions with poor performance (<70% overall or fewer than 8 correct memory trials without fixation violations per side)

were excluded from analyses. These sessions were rare (2.4% of all "final stage" sessions) and were likely caused by problems with the hardware (e.g. a clogged water-valve or a dirty IR-photodetector).

Surgery

All surgeries were done under isoflurane anesthesia (1.5-2%) using standard stereotaxic technique. Rats were given an injection of ketamine (10 mg) and buprenorphine (0.006 mg) to assist induction and provide analgesia. Five minutes later they were placed in an isoflurane induction chamber. We slowly increased the concentration of isolflurane from 0.5% to 4% over the course of 4 minutes. After induction, rats were moved to a stereotax (Kopf Instruments; CA) and their noses placed in a cone which provided 1.5-2% continuous isoflurane flow. After verifying surgical levels of anesthesia with pinch tests and eye blink tests, rats were secured in non-rupture ear bars (Kopf Instruments; CA). The scalp was shaved and cleaned with ethanol, betadine and ethanol and then a midline incision was made with a scalpel. A spatula was used to clean the skull of all overlying tissue before craniotomies were made with a dental drill over the FOF (AP +2, ML ±1.3 mm from Bregma). This location was chosen because it was the center of the distribution of stimulation cites that resulted in contralateral orienting orienting movement in Sinnamon & Galer (1984). Durotomies were then performed. After the durotomies were finished, saline soaked Gelfoam (Pfizer Injectables; NY) was placed in the craniotomies to protect the brain for the next step. Then a thin coat of C&B Metabond (Parkell, Inc; NY) was painted all over the skull. The Gelfoam was then removed from the craniotomies and the implant (either electrodes or cannula) was lowered onto the brain surface. For electrode implants only the tetrodes entered the cortex. For cannula implants (Plastics One, VA) the outer cannula was placed at brain surface and the injector (inserted only during infusions) extended 1.5 mm past the end of the guide. DuraLay (Reliance Dental, IL) cement was used to secure the implant to the Metabond coated skull. Rats were given buprenorphine and ketofen 24 and 48 hours post-operative and were allowed to recover on ad lib water for 5 days before returning to water restriction and behavioral training.

Subsequent histological verification and comparison to Paxinos and Watson's atlas, which is based on Wistar strain rats (Paxinos and Watson, 2004), indicated that our implants were at a position matching +2.4 AP, ±1.3 ML in the atlas. The target of the neck M1 surgeries was +3.5 AP, +3.5 ML (Gage et al., 2010).

Infusions

Five rats were used for the infusion experiments in FOF and 4 rats were used for the infusion experiments in M1. Rats were placed under a light non-surgical 1-1.5 % isoflurane anesthesia for infusions. The cap and dummy cannula were removed and replaced with an injector that extended 1.5 mm beyond the end of the guide cannula. For the FOF dose and volume of muscimol was 0.5 mg/mL and 0.3 uL respectively. For M1 we first used the same dose and volume as in the FOF (0.3 uL of 0.5 mg/mL muscimol) and we followed up that experiment with double the dose (0.3 uL of 1 mg/mL muscimol). The rate of infusion was 0.2 uL/min and we waited 4 minutes after infusions before removing injectors to allow the drug to spread. Rats were allowed to recover for 30 minutes from the anesthesia before beginning their training sessions for that day. Rats were placed in and removed from the behavior box by technicians that were blind to the task and the side of the infusion. Each infusion day was followed by a minimum of 3 days of running with no infusions. Left and right infusions were alternated. Each rat received at least two right infusions and two left infusions.

For analysis of the effects of unilateral muscimol infusions we compared infusion days with the days immediately preceding the infusion. For example, if we did infusion on the 10th and 20th of the month, then we would use data from the 9th and the 19th as control days. We did not use saline infusions as a control because we are not specifically interested in the mechanism of the effect of muscimol, but rather whether perturbing unilateral function of the FOF has a lateralized effect on performance. In addition, we argue that the right and left infusions act as a control for each other, since in both cases the animals experience the same procedure, the only difference being the side of the infusion. Thus, any behavioral difference between left and right infusions would be due to the drug infusion and not some other aspect of the infusion procedure (a change in handling or the isoflurane).

Whisker Experiments

In order to determine the contribution of whisking to memory-guided orienting we conducted three experiments: Unilateral lidocaine injections into the whisker pad; video analysis of the whiskers during the task; and removal of the whiskers. For the unilateral lidocaine experiments rats were anesthetized with isoflurane and then 0.2 cc of 1% lidocaine was injected subcutaneously either at the right or left whisker pad. The front and rear claws were blunted with a nail file to prevent self-injury from scratching at the anesthetized site. The effect of lidocaine was visually confirmed by unilateral absence of whisking on the injected side before training and every hour thereafter. In every case the effect of lidocaine lasted for at least 140 minutes. In some cases the whiskers were marked with titanium dioxide paint (Sigma-Aldrich, Product # 224227 mixed with clear nail polish) to facilitate visualization of the whiskers on video. The injections were performed on alternate sides with no-injection days in between each experiment: for example, a right injection on the 10th, a left injection on the 12th and a right injection on the 14th.

For video analysis of whisking during memory guided orienting we used small pieces of aluminum foil glued to the whiskers as well as beads of titanium dioxide paint to make the whiskers more visible on video. Video was collected at 29.97 frames / sec using a Hamamatsu CCD (XC-77). Illumination was provided by an infra-red led 30Hz strobe light (custom made) with a 30% duty cycle to reduce motion blur. Then the position of the head and whiskers were marked by hand on each frame (assisted by a custom algorithm based on the local cross-correlation between subsequent frames). We used the distance of the whisker marker to the head marker as a measure of whisker position. We then used a multi-taper method (pmtm, MATLAB) with a WM (time-bandwidth product) of 2 to determine the spectral content of the whisker movements separately for exploratory whisking and delay period whisking on left versus right correct memory trials (**Figure S1**).

Recordings

Five rats were used to collect single-unit electrophysiology data. Recordings were made with platinum iridium wire (16.66 μm, California Fine Wire, CA) twisted into tetrodes. Each tetrode was threaded into an polyimide tube (34 AWG triple wall) which was part of a movable bundle of eight tubes. Two rats were implanted with a movable bundle of 8 tetrodes on each of the right and left FOF (16 tetrodes per rat). Three rats were implanted with an 8-tetrode bundle unilaterally. Implants were targeted to +2 AP ±1.3 ML (mm relative to Bregma). Within each bundle, tetrodes were spaced ~250 μm from each other; we estimate that neurons were sampled from within a radius of ~0.5 mm. Wires were gold-plated to 0.5-1.2 MOhm. The tetrodes could be advanced by turning a nut against a spring on a 0-80 threaded rod so that a 1/8 turn drove the tetrodes down about 40 μm. The tetrodes were advanced at the end of sessions so that the brain tissue had time to stabilize before recording the next day.

We used two electrophysiology systems to collect the data presented here. In both cases the reference selection, analog to digital conversion, time-stamping, filtering (600-9000Hz, FIR filter) and video tracking was done using a Digital Cheetah System (Neuralynx; MT). For some recording we used Neuralynx unity gain headstages (HS-36, Neuralynx). For other recordings we used 4 x 31 channel (124 channel total) time-division multiplexing headstages (Triangle BioSystems Inc, NC) with a 10 channel commutator (Dragonfly, WV), and then the signals were demultiplexed and then used as input to the Digital Cheetah system.

Spike sorting was done by hand using SpikeSort3D (Neuralynx). Cells had to satisfy several criteria to be included in the presented analyses: 1) No inter-spike intervals < 1 ms; 2) Signal to noise ratio >4; 3) At least one bin of the perivent time histogram (PETH, aligned to the Response Onset time), had to have a firing rate of at least 3 spikes/sec. The PETH was a 2 second window (-1.5s before Response Onset to 0.5s after) with 10 ms bins and a causal half-gaussian smoothing window with a s.d. of 200 ms (effective smoothing was 100 ms since only half the gaussian was used). 378 cells recorded over 100 sessions satisfied the first two criteria (i.e. Well-isolated single units). 242 cells satisfied all three criteria. Median number of cells per session was three. The maximum number of cells recorded in a session was eleven.

Behavior Analysis

To generate the psychometric curves (**Figure 1b,c**) we analyzed all sessions from the final training stage for each rat. Each session generates 12 behavioral data points: the % "Went Right" for each frequency (n=6) for both memory and non-memory trials. We then combined the data across sessions (separately for memory and non-memory trials) and fit that data with a 4-parameter sigmoid where **y**, "% went-right" is a function of **x**, the log(click frequency):

$$y = y_0 + \frac{a}{1 + e^{\frac{-(x - x0)}{b}}}$$

The four parameters are: $\mathbf{y_0}$, the lower asymptotic performance; $\mathbf{y_0+a}$, the upper asymptotic performance; $\mathbf{x_0}$, the location in log(frequency) of the inflection point of the curve; **b** , the slope of the curve. If a rat ran 100 sessions we would fit 4 parameters to 600 data points for memory trials and another 4 parameters to 600 data points for non-memory trials. Fitting was done with Matlab's nlinfit.

For analysis of the effects of whisker trimming we included data from the day of trimming and the two following days. (It takes several weeks for whiskers to grow back to their normal length.) We generated psychometric fits to the whisker trimming data in the same way as we did for the behavioral data in **Figure 1b,c.** Since the whisker trimming experiment immediately followed the lidocaine experiments we used the same sessions as controls for the lidocaine and the whisker trimming sessions.

We generated the psychometric fits to the muscimol and control data in the same way as for **Figure 1**. To generate statistics for the effect of muscimol we combined left and right infusion days across rats and labeled trials as contra- or ipsi-muscimol. For example, if we infused into the left FOF then trials where the correct response was toward the right would be labeled as contra-muscimol for that session. To determine statistical significance we bootstrapped the distribution for the mean of the differences between conditions. We did 8 tests: memory vs. non-memory for ipsi-muscimol trials; memory vs. non-memory for contra-muscimol trials; ipsi-muscimol vs. contra-musicmol for non-memory trials and ipsi-muscimol vs. contra-muscimol for memory trial; ipsi-muscimol vs. control for non-memory trials and ipsi-muscimol vs. control for memory trials; contra-muscimol vs. control for non-memory trials and contra-muscimol vs. control for memory trials. The p-values reported are the probability that the mean difference is zero for each comparison. The analyses and statistics for the M1 muscimol experiments were identical to the FOF experiments.

Neural Data Analysis

To determine whether cells had upcoming-choice-dependent firing rates during the delay period on memory trials, we determined the firing rate of the cells during the delay by counting the number of spikes fired after the offset of the auditory stimulus and before the Go cue; we then divided by the duration of the delay period on that trial to obtain the firing rate. We sorted the trials into correct left and correct right trials. We then computed the area under the receiver operator characteristic curve (AUC) for the distribution of firing rates on left vs. the distribution of firing rates on right trials. To determine statistical confidence on the AUC value we randomly relabeled the trials as left or right (keeping the # of left and right trials the same) and computed the AUC of the shuffled trials. We did this 2000 times. A cell was considered significantly side selective if the AUC of the data was outside the 95% confidence intervals of the shuffled data.

To determine at which timepoints cells had upcoming-choice-dependent firing rates, we first generated single trial firing rate traces by convolving the spike trains on each trial with a causal half-Gaussian (s.d. of 200 ms) smoothing kernel. At each timepoint, running in 10 ms bins from 1.5 seconds before the Go cue to 0.5 seconds after the Go cue, we then computed the AUC of the distribution of left vs. right correct firing rates. To compute the significance for each cell at each time bin we randomly relabeled the trials as left/right and computed the AUC for the shuffled data 1000 times. If the AUC of the data for a given neuron at a given time bin was outside the 99% confidence interval of the shuffled data then that time bin, for that cell, was labelled as significant. We then determined, for each cell, the largest number of time bins labeled as significant in the shuffled data (n) that would result in 5% or more of the shuffled trials having n or more significant time bins. (The larger that n is, the fewer the shuffled

trials that are labeled as significant.) If the original data for the cell had more than n time bins labeled as significant, then the cell as a whole was labeled as significant at $p<0.05$.

In order to quantify the timing of the recruitment of the FOF population over the course of the trial we used the same sliding ROC analysis of left vs. right correct memory trials with activity aligned to the time of the Go cue as above. According to binomial statistics, using a threshold of $p<0.01$, we would expect less than 8/242 cells to be significant by chance 99.9% of the time.

To perform population analyses of firing rates, we first normalized the perievent time histograms (PETHs) of each cell by computing the mean and standard deviation (over time and over trial classes) of the cell's PETHs, and then subtracted that mean and divided by that standard deviation. The resulting z-scored PETHs were then averaged across cells to obtain z-scored population PETHs.

In order to quantify whether neurons in FOF tended to encode the stimulus or the response we generated a Stimulus Selectivity Index (SSI) from Go aligned PETHs for correct and error trials as follows:

$$SSI_{tt} = \frac{\sum_{t=-1.5}^{0.5} PETH_{contra,tt} - PETH_{ipsi,tt}}{\sum_{t=-1.5}^{0.5} PETH_{contra,tt} + PETH_{ipsi,tt}}$$

If a cell fired only on contra and not on ipsi trials, then SSI=1. If a cell fired on ipsi and not contra trials, then SSI=-1. If a cell fired equally for ipsi and contra trials then SSI=0. We computed an SSI for the following four trial-types (*tt*):correct-memory, correct-non-memory, error-memory, and error-non-memory. The contra/ipsi label referred to the instructed response for that trial. So for error trials the $PETH_{ipsi}$ was constructed from trials where the rat was instructed to go *ipsi* but instead went *contra*. We also calculated delay interval [-1.5 to 0 sec relative to Go] and response interval [0 to 0.5 sec relative to Go] SSIs.

We computed the choice probability of neurons in order to quantify the covariance of neural activity during the delay to variability to behavior. We counted spikes in a 400ms ending with the Go cue on memory trials where the rat was instructed to respond towards the neurons preferred side. We used ROC analysis to determine how well the firing rate in that window predicted the future choice of the rat.

Latency Analysis

We used an alignment algorithm to find a relative temporal offset for the neural and behavior data on each trial as follows. Single-trial PETHs were generated as described previously. Then a trial-averaged PETH was generated for each cell. For each trial we found the time of the peak of the cross-correlation function between the PETH for that trial and the trial-averaged PETH. We then shifted each trial accordingly and iterated this process until the variance of the trial-averaged PETH converged. Usually this process required fewer than 5 iterations. The output of this alignment procedure was an offset time for each trial, which indicated the relative neural latency for that trial. We performed the same alignment procedure on head-velocity data acquired with the video-tracking system, which produced a relative behavioral latency for each trial. We then tested whether the neural latency was correlated with the behavioral latency and whether for the population the average correlation was significantly different than zero (Bootstrapped confidence intervals of the mean). We also compared, in the same way, the neural latencies of pairs of simultaneously recorded neurons.

Head Direction Tuning Analysis

We determined the relationship between firing rate and head-direction in and out of the task in the following way. "In-task" epochs were defined as any time in the session within 10 seconds of the rat poking in any nose port. "Out-of-task" epochs were defined by exclusion as the times the rat was not in-task. The head-direction for each epoch was divided into eighteen 20 degree bins, with the center port aligned to 0 degrees. Each contiguous stretch of time, in which the head direction lay within a single direction bin, was taken as a single data point. Firing rate for that data point was simply the number of spikes fired divided by time duration. Average firing rate for each direction bin was obtained by averaging the firing rates for all data points for that bin; error bars are the bootstrapped 99% confidence intervals of the mean. Occupancy was defined as the number of data points that fell within each bin. Having thus

obtained firing rate as a function of head direction, we then computed a summary vector **v** for each cell. Each cell's summary vector was the weighted average of a set of unit length vectors, each of which pointed in each of the eighteen different head direction bins. The weighting was proportional to the cell's firing rate in each direction bin. Thus the vector **v** for each cell was

$$\mathbf{v} = \frac{1}{\sum_b f_b} \sum_{b=1}^{b=18} f_b \hat{\mathbf{u}}_b \qquad \hat{\mathbf{u}}_b = [cos(\theta_b) \quad sin(\theta_b)]$$

where f_b is the firing rate for direction bin b, and θ_b is the angle that corresponds to direction bin *b.* For example, if a cell fired equally at every head direction, then **v** would be a vector with zero length. If a cell fired only at 40˚ and nowhere else then **v** would be a vector with length 1 and an angle of 40˚.

Regression Analysis

To asses the extent to which delay period activity was simply a function of movement parameters, we modeled the firing rate of each cell from [-1.5s to 0.5s] relative to the Go cue on correct memory trials as a linear function of head angle, angular velocity and angular acceleration:

$$f(t) = \beta_1 \cdot \phi(t) + \beta_2 \cdot \phi'(t) + \beta_3 \cdot \phi''(t) + \beta_4 + r(t)$$

Where f(t) is the PETH of each cell. If the cell's firing rate were a linear function of movement parameters then the residuals r(t), would on average be zero. However, if there the firing rate encoded the future choice of the rat on a trial, separately from the head direction parameters, then the linear model would not fully account for f(t) and information about the future choice would remain in r(t). We performed ROC analysis on the residuals r(t), at each timepoint for left and right trials in the same way as the analysis on firing rate for **Figure 3C**. The results are shown in **Figure S7I**.

Histology

Histology was performed on the cannula implanted rats (**Figure S2E-H**) and electrode implanted rats (**Figure S3C,D**) once all experiments were completed. In all cases the FOF placements were within the borders of M2 and between 2 and 3 mm anterior to Bregma. In all cases the M1 placements were within the borders of M1 and between 2.5 and 3.5 mm anterior to Bregma (Paxinos and Watson, 2004).