Supporting Information

SI Appendix

Animal experimental setup. The protocols used in this study were approved by Queen's University Animal Care Committee in accordance with the Canadian Council on Animal Care policies on the use of laboratory animals. Experiments were performed on two male rhesus monkeys (*Macaca mulatta*; 11 and 12 kg). The methods of surgical procedures, techniques for extracellular neuronal recording and data collection have been described in detail previously (1). Eye position and pupil size were measured by a video-based eye tracker (Eyelink-1000, SR Research, Osgoode, ON, Canada) at a rate of 1000 Hz with monocular recording. Stimulus presentation and data acquisition were controlled by a UNIX based real-time data control system (REX) (Hays et al., 1982). Spikes, eye position, and pupil diameter were recorded in a multichannel data acquisition system (Plexon). Stimuli were presented on a CRT monitor at a screen resolution of 1024 x 768 pixels (75Hz non-interlaced), subtending a viewing angle of 54 x 44 deg. One of the monkeys (monkey B) was found to have strabismus during the course of data collection, the vision of his misaligned eye (right) was covered by a black board attached to the chair to avoid potential double vision.

Procedure, SC microstimulation and microinjection. Monkeys were seated in a primate chair with their heads restrained facing the video monitor. We lowered tungsten microelectrodes (impedance: 0.1–1 MΩ, Frederick Haer) to determine the depth of the SC. Once the SC had been located by single neuron recording and the visual response fields were mapped using a visual mapping task (2), monkeys performed a delayed saccade task to confirm the presence of motor activity. Each trial started with fixation of a central fixation spot (0.5° diameter, 30 cd/m^2)

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against a black background for 500–800 ms, and then a target stimulus (0.5° diameter, 30 cd/m^2) appeared in the response field of the neuron. After a delay (500–800 ms), the fixation spot was removed and the monkey was required to generate a saccade toward the target. Because target presentation was temporally dissociated from the saccade, the visual and the motor components of the discharge were isolated and easily distinguished. Once the SCi was confirmed (neurons with pronounced increases in discharge related to the initiation of saccades(3–5)), the SCi was microstimulated (300 Hz pulse train for 100 ms with alternating 0.3 ms anode plus 0.3 ms cathode pulses) and threshold for saccades was determined when the stimulation current in the SCi evoked saccades 50% of the time (range: 5-50 μA). The optimal locations of the response fields of SCi neurons were in close agreement with the vector of eye movement elicited with suprathreshold SCi stimulation, and the center of one of the patch stimuli was placed at the region of the field to which the saccade was directed. After determining the location of the stimulation site by evoking saccades, we further reduced the frequency of stimulation from 300 to 70-90 Hz and used 25-45% of the saccade threshold current to activate the target area in the SC without evoking either saccades or pupil dilation (6, 7).

Microinjections of lidocaine or saline were made through a metal cannula with an attached microelectrode. Injections consisted of 1–1.3 μl of 2% lidocaine or saline alone were delivered at a rate of 0.5 μl/min using a Hamilton syringe into the SCi at a depth that was 1–2.5 mm below the SC surface according to the history of microelectrode recordings. Furthermore, saccades were reliably elicited at low microstimulation currents at the depth of injection (less than 30 μA at 300 Hz). Testing before (pre-injection), after injection (post-injection: ~1-20 mins after the injection), and after recovery (recovery: 25+ mins after the injection) was usually conducted in the same session, recovery testing was occasionally conducted on the next day.

Behavioral paradigms. *Fixation and microstimulation task (Exp 1-3).* Monkeys were trained to perform fixation tasks. In Experiment 1 (Fig. 1a*)*, they had to maintain gaze within 1.5° of a fixation point (FP, 0.5° diameter; 20 cd/m^2, isoluminant color of the background) at the center of the screen on a gray background (20 cd/m^2) for a few seconds to obtain a liquid reward. After the monkey maintained fixation for 1–1.5 s, a train of stimulation pulses was delivered (400 ms, 70-90 Hz, 25-45% saccade threshold) on 50% of the trials coincident the presentation of two task-irrelevant patch stimuli (3-9° in radius, patch size varied relatively according to eccentricity-larger diameter with larger eccentricities; one bright and the other dark, both with 95% contrast relative to the gray background). Monkeys had to maintain fixation for another 1.5–2.5 s regardless of microstimulation (note that 3 sites in Experiment 2 used 100 or 200 ms microstimulation, and effects were similar to those obtained when we used 400 ms of microstimulation). Two patch stimulus conditions were used: in the bright condition (50% of trials), the center of the bright patch stimulus location was spatially aligned with the SCi stimulated location determined by the saccade vector evoked via suprathreshold SCi microstimulation, and the dark patch was presented at the opposite location of the bright patch. In the dark condition (50% of trials), the center of the dark patch stimulus location was spatially aligned with the SCi stimulated location, and the bright patch was presented at the opposite location of the dark patch. Experiment 2 was identical to Experiment 1, except for introducing four configurations of the patch stimuli relative to the SCi site: the patch stimuli were presented either aligned and opposite the SCi stimulation site (Fig. 2a), or orthogonal to this configuration (Fig. 2b). In Experiment 3 (Fig. 3a), two patch stimuli were displayed at the fixation onset and microstimulation was delivered 1000-1500 ms later. All conditions were randomly interleaved.

Microstimulation was delivered to 32 sites in Experiment 1 (21 and 11 in monkeys A and B, respectively), to 14 sites in Experiment 2 (7 and 7 in monkeys A and B, respectively), and to 25 sites in Experiment 3 (16 and 9 in monkeys A and B, respectively). The optimal locations of the response fields determined by suprathreshold microstimulation (ranged from between 10° and 25° eccentricity) at these sites. It is important to note that the patch stimuli were task-irrelevant, so the monkey should completely ignore those stimuli to perform the task correctly. Moreover, to prevent the monkey simultaneously from attending both patch stimuli, the patch stimuli were presented outside the foveal region of central fixation (beyond 5° in radius of FP).

Injection task (Exp 4). Monkeys were trained to perform a saccade task (Fig. 4a) that required a saccade made toward the injected location or opposite of it after presentation of a visual target with FP disappearance to examine the saccade behavior influenced by the injection (not displayed in Figure). After monkeys maintained fixation within 1.5° of a FP (0.5° diameter; 18 cd/m^2) on a gray background (20 cd/m^2) for 0.8–1 s, the two patch stimuli were presented for 200 ms (3-7° in radius; one bright and the other dark, both with 95% contrast relative to the gray background, 57% of trials), and then monkeys had to maintain fixation for another 1–1.2 s before the removal of the FP with the presentation of a visual target (0.5° diameter; ~15 cd/m^2), requiring monkeys to generate a saccade toward the target to obtain a liquid reward. Moreover, a no-stimulus condition was added (43% of trials) and no patch stimulus was presented to normalize pupil diameter in the different periods of injection (pre-injection, post-injection, recovery). A visual target was spatially aligned with the injection location, or was presented at the opposite location of the injection location. A total of 8 injections was made in two monkeys, 6 lidocaine and 2 saline injections (3+1 in monkey A and B).

Visual-delayed and memory-guided saccade tasks (Exp 5). Monkeys were trained to perform the visual-delayed and memory-guided saccade tasks (Fig. 5a). Monkeys had to maintain gaze within 1.5° of a central FP (0.5° diameter; 20 cd/m^2, isoluminant color of the background) on a gray background (20 cd/m^2) for 0.8–1.2 s. A target stimulus (0.5° diameter, isoluminant color of the background) then appeared at one of four different radial angles in each block (0, 90, 180, 270° or 45, 135, 225, 315°) at an eccentricity of 10-15° visual angle from the central FP. In the memory-guided task, the target was flashed for 100 ms. After a delay (500–900 ms), the two patch stimuli were presented for 400 ms (3-6° in radius, one bright and the other dark, both with 95% contrast relative to the gray background; in the visual-delayed task, target was removed simultaneously, see Fig. 5a), and then the monkey had to maintain fixation for another 1–1.5 s before the removal of the FP, requiring the monkey to generate a saccade toward the target to obtain a liquid reward. The patch stimuli were presented either aligned and opposite the target location (Fig. 5b, 18.75% of trials for each bright and dark condition), or orthogonal to this configuration (Fig. 5c, 18.75+18.75 % of trials). Note that control visual-delayed and memory-delayed conditions were added (no patch stimuli, not displayed in the figure) as filler trials (25%) to prevent the monkey from developing a particular strategy to perform these tasks (e.g., not actively preparing a saccade towards the target during an initial delayed period). In this case, the FP was removed after 500-800 ms of target presentation. The visual-delayed and memory-guided tasks were performed separately, and trials in each task were collapsed across 2-5 daily full recording sessions. A total of ~2200 (Monkey A: 700; Monkey B: 1500) and ~1950 (Monkey A: 650; Monkey B: 1300) correct trials were recorded from the visual-delayed and memory-guided tasks, respectively.

Data analysis. To maintain an accurate measure of pupil size, trials with an eye position deviation of more than 2° from the central FP or with detected saccades (> 2°) during the required period of central fixation were excluded from analysis. To better explain the characteristics of change in pupil size (8, 9), we followed the procedures of baseline-correction used previously (6, 10). For each trial, a baseline pupil value was determined by averaging pupil size during the epoch 200 ms to 50 ms before the onset of electrical stimulation because the pupil response latency is greater than 100 ms. Pupil values were subtracted from this baseline value. Monkeys may preferably attend to one visual field or may be biased to the stimulated location even on the trials without microstimulation. To avoid these biases, we contrasted the normalized pupil diameter values between the stimulation versus no-stimulation conditions directly. The average value of the normalized pupil diameter during two time windows were selected to capture the pupillary changes modulated by microstimulation for different fixation tasks (Fig. 1-3), epoch of 300 to 600 ms after the stimulation onset for Experiment 1 or 2, and epoch of 200 to 500 ms after the stimulation onset for Experiment 3 (this epoch was earlier than the previous one because patch stimuli were presented well before the time of microstimulation. Thus the effects of microstimulation on the patch stimuli were revealed faster). In the injection experiment, after baseline-correction, pupil diameter in the no-stimulus condition was subtracted from the bright or dark patch condition to minimize an arousal influence on pupil size across recording periods (pre-injection, post-injection, recovery). Moreover, as mentioned previously, monkeys may preferably attend to one visual field. To eliminate this bias, we contrasted the normalized pupil diameter values between the post-injection versus pre-injection or recovery period to directly examine the effects of injection on the local luminance modulation. The suggested method was used to transfer output pupil area values recorded from the eye tracker to actual pupil size in

diameter (11). Cohen's *d* was calculated manually or using a matlab tool box (12) to estimate the effect size. To specifically examine our hypothesis that pupillary responses evoked by patch stimuli should be smaller when the bright patch stimulus (compared to dark) was spatially aligned the stimulated SC location (and the reversed prediction pattern for the lidocaine injection experiment), we performed a one-sided *t* test, except where indicated.

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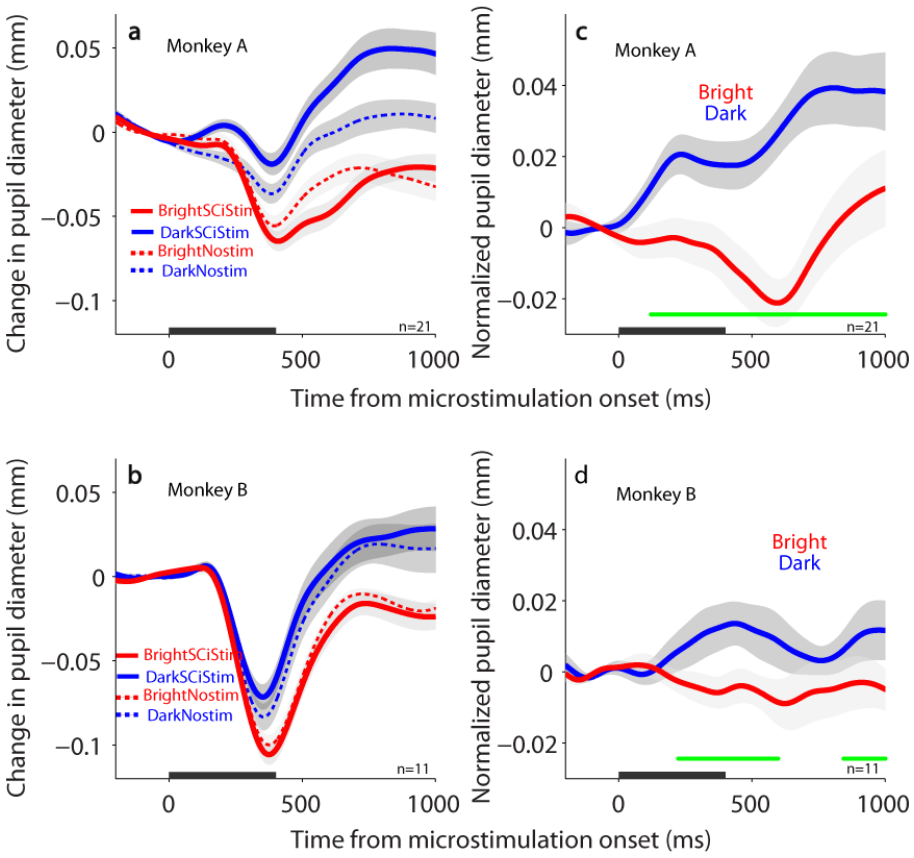
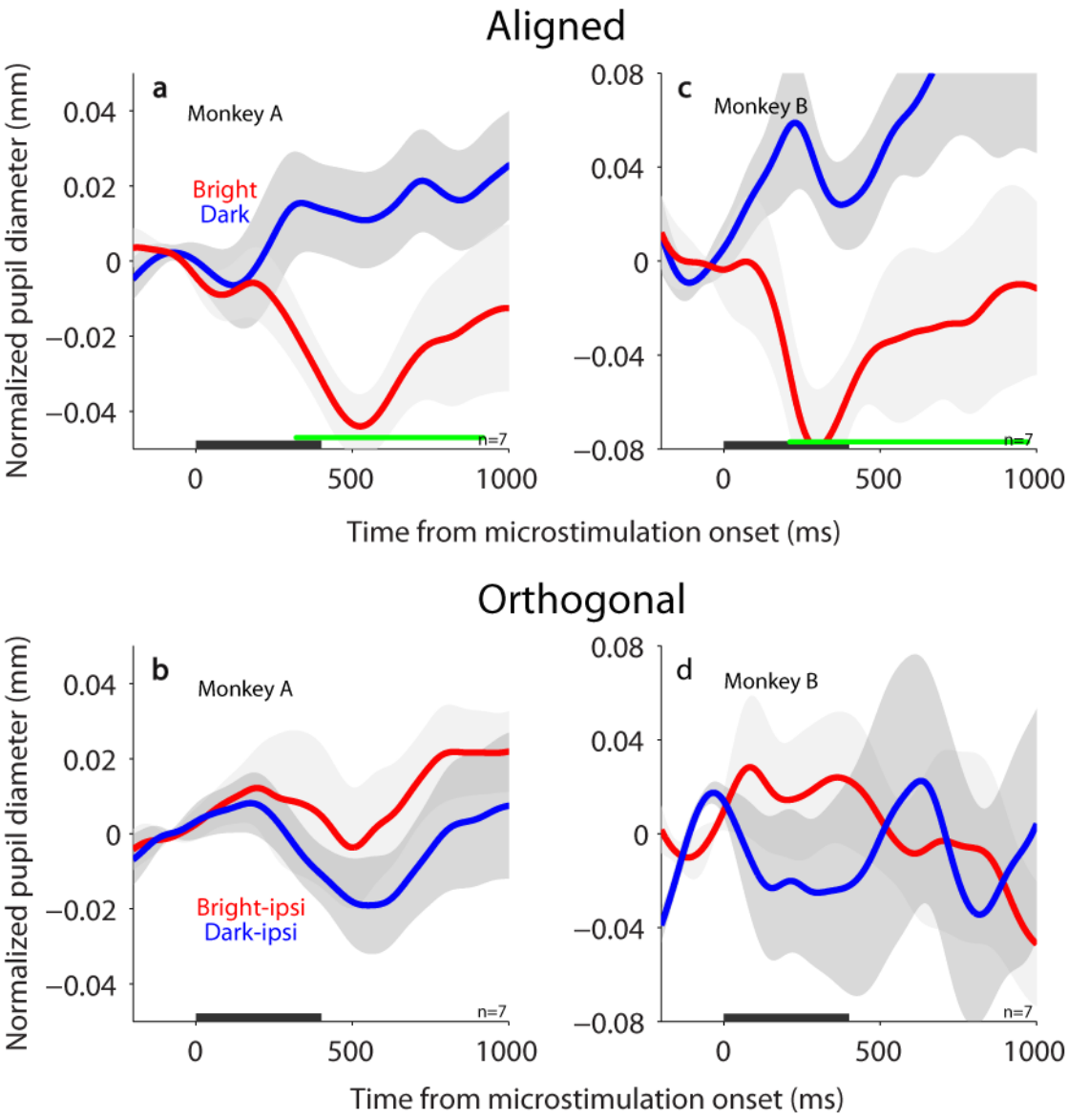
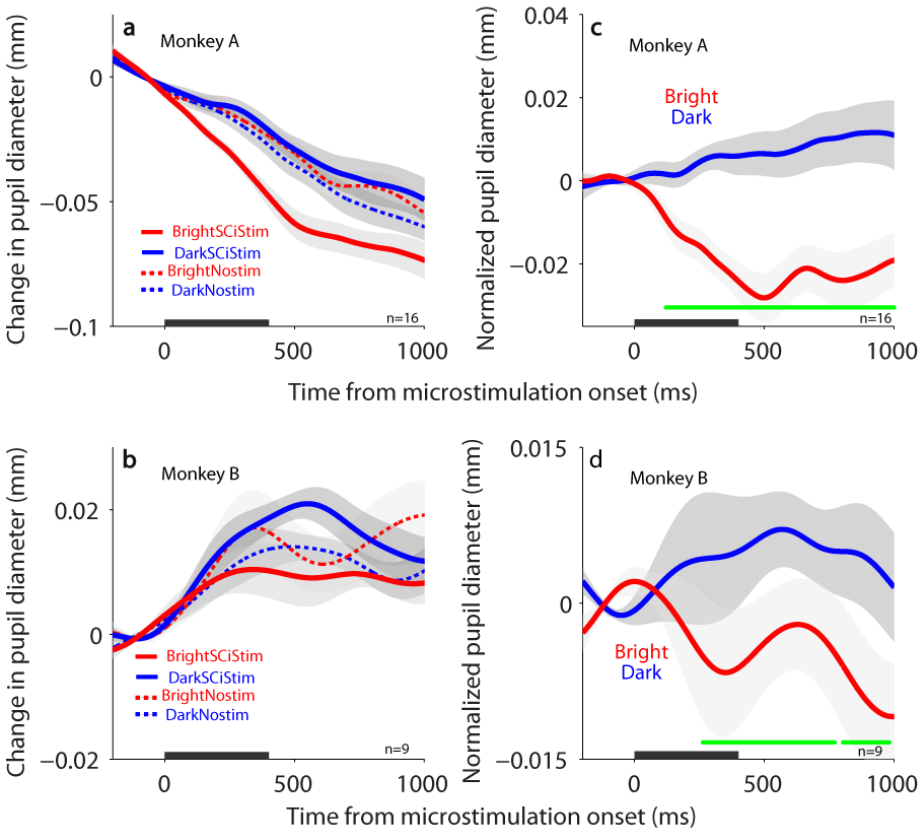


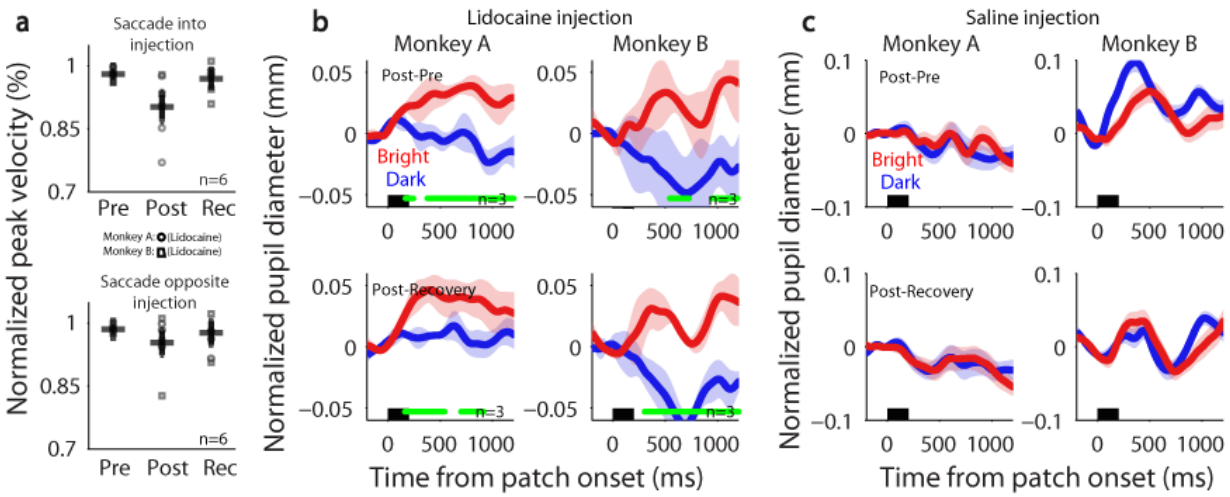
Supplementary Figure 1. Effect of SCi microstimulation on local luminance modulation in each monkey. (a,b) Effects of SCi microstimuation on pupil size following the patch presentation in (a) monkey A (n=21) and (b) monkey B (n=11). (c,d) The normalized pupil diameter (differences between microstimulation and no-stimulation trials) in the bright and dark condition in (c) monkey A (n=21) and (d) monkey B (n=11). The black bar on X-axis indicates the time line of microstimulation. The shaded regions surrounding the pupillary response represent ± standard error range (across sites) for different conditions. The green bar on X-axis indicates the time line at which differences between the bright and dark conditions were statistically significant ($p < 0.05$). n: number of sites. Note that the bias towards stimulation location on no-stimulation trials was present at 24 of 32 stimulation sites. Although pupil dynamics induced by patch stimuli were slightly different between two monkeys, they followed a similar pattern after the patch presentation: "subtle" dilation followed by clear constriction. More importantly, they both showed the same modulated responses by microstimulated patch luminance.



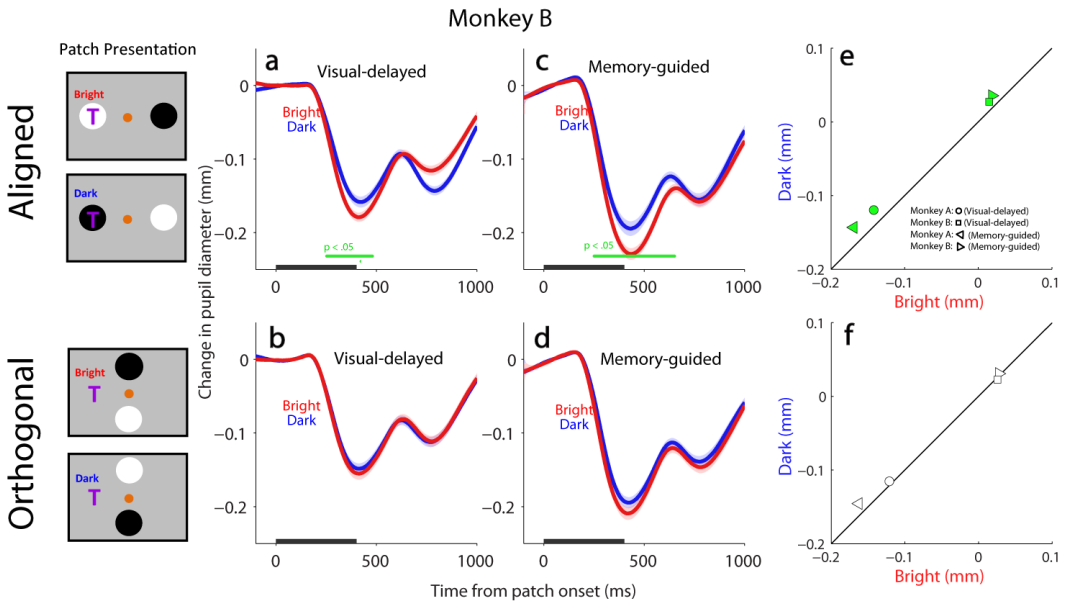
Supplementary Figure 2. Effect of SCi microstimulation on different patch location conditions in each monkey. (a,b) Effects of SCi microstimuation on the normalized pupil diameter (differences between microstimulation and no-stimulation trials) in the bright and dark condition in (a) monkey A (n=7) and (b) monkey B (n=7). (c,d) Effects of SCi microstimuation on the normalized pupil diameter in the ipsi-lateral (of the stimulation site) bright and dark condition in (c) monkey A (n=7) and (d) monkey B (n=7). The black bar on X-axis indicates the time line of microstimulation. The shaded regions surrounding the pupillary response represent ± standard error range (across sites) for different conditions. The green bar on X-axis indicates the time line at which differences between the bright and dark conditions were statistically significant ($p < 0.05$). n: number of sites.



Supplementary Figure 3. Effect of SCi microstimulation on local luminance modulation in each monkey in the second fixation experiment. (a,b) Effects of SCi microstimuation on pupil size following the patch presentation in **(a)** monkey A (n=16) and **(b)** monkey B (n=9). **(c,d)** The normalized pupil diameter (differences between microstimulation and no-stimulation trials) in the bright and dark condition in **(c)** monkey A (n=16) and **(d)** monkey B (n=9). The black bar on X-axis indicates the time line of microstimulation. The shaded regions surrounding the pupillary response represent ± standard error range (across sites) for different conditions. The green bar on X-axis indicates the time line at which differences between the bright and dark conditions were statistically significant ($p < 0.05$). Stim: microstimulation, NoStim: no microstimulation. n: number of sites. Notably, pupil dynamics were very different between two monkeys (one constricting and another dilating), and this could be due to the simple task requirement of central fixation, and therefore different monkeys may have a very different mindset during the task. Importantly, though different pupil dynamics, both monkeys showed the same modulated responses by microstimulated patch luminance, suggesting that the observed effects were indeed reliable.



Supplementary Figure 4. Effect of microinjection on local luminance modulation in each monkey. (a) Effect of injection on the peak velocity for saccades made to the injection location or opposite of the location during pre-injection, post-injection, or recovery period. Saccade velocity was divided by the median of saccade velocity in the pre-injection period to normalize the data. **(b)** The normalized pupil diameter with lidocaine injection (differences between post-injection and pre-injection or recovery period) in the bright and dark condition in monkey A (n=3) and monkey B (n=3). **(c)** The normalized pupil diameter with saline injection (differences between post-injection and pre-injection or recovery period) in the bright and dark condition in monkey A (n=1) and monkey B (n=1). In **a**, the cross and error-bar represent mean with ± standard error range (across sites, n=6). In **b-c**, the black bar on X-axis indicates the time line of microstimulation. The shaded colored regions surrounding the pupillary response represent ± standard error range (**b**: across sites; **c**: within site) for different conditions. The green bar on X-axis indicates the time line at which pupil size in the bright condition were statistically significant larger than in the dark condition ($p < 0.05$). Saccade into injection: saccades made to the injection location. Saccade opposite injection: saccades made to the opposite of the injection location.



Supplementary Figure 5. Effect of saccade planning on local luminance modulation in monkey B. (a,**c)** Change in pupil diameter following the presentation of patch stimuli in the bright and dark aligned conditions in **(a)** the visual-delayed task and **(c)** the memory-guided task. **(b**,**d)** Change in pupil diameter following the presentation of patch stimuli in the bright and dark orthogonal conditions in **(b)** the visual-delayed task and **(d)** the memory-guided task. **(e-f)** Summary of behavioral effects between the bright and dark conditions across monkeys and tasks on **(d)** aligned, and **(e)** orthogonal conditions. In **a-d**, the shaded colored regions surrounding the pupillary response represent ± standard error range (across trials) for different conditions. The gray bar on X-axis indicates the time line of patch presentation, and the green bar on X-axis indicates the time line at which differences between the bright and dark conditions were statistically significant ($p < 0.05$). In **e**,**f**, filled-areas indicate statistically significant differences ($P < 0.05$).T indicates the target location.