Selective Attention from Voluntary Control of Neurons in Prefrontal Cortex

Robert J. Schafer, *et al.*

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with standard blue-light pulses (the control group was not exposed to blue-light pulses). shGLP-1 (Fig. 5A) and the resulting insulin (Fig. 5B) levels were significantly increased in wild-type as well as in diabetic db/db mice after blue-light exposure, and the action of both proteins significantly reduced the glycemic excursion of treated animals after intraperitoneal administration of glucose (wild-type mice, Fig. 5C; diabetic db/db mice, Fig. 5D). On the basis of these glucose tolerance tests showing the improvement of glucose homeostasis, and recent reports suggesting that the glucose-dependence of shGLP-1 automatically shuts down insulinotropic actions upon reaching normal glucose levels and so prevents hypoglycemia (*25*), light-triggered expression of shGLP-1 may be considered for the treatment and prevention of glucose-related pathologies (*25*, *26*).

From plants to mammals, light-based energy and information is captured via receptors and processed via ion-based membrane potential (*2*, *3*, *27*, *28*). Some of these native light receptors, such as melanopsin (*3*) and channelrhodopsin (*29*), have been extensively used for heterologous intervention in native neuron-triggered activities in order to understand the photoentrainment of the circadian clock (*7*) or to restore visual function in retinal degeneration (*5*, *6*, *29*, *30*). Capitalizing on the principles of synthetic biology to assemble functional biologic devices from well-characterized components in a rational and predictable manner (*31*), it has recently become possible to engineer synthetic signaling cascades and control networks to program metabolic behavior (*32*–*35*), cell morphology (*36*), and therapeutic interventions (*37*) with high precision. By combining heterologous factors (melanopsin) and control modules (P_{NFAT}) with promiscuous complementary endogenous machineries (G proteins, NFAT pathway), we rewired melanopsin-mediated G protein–coupled receptor signaling to NFAT control, taking advantage of their common intracellular calcium-based, second-messenger–based signaling system as the interface (*16*). When engineered into mammalian cells grown in bioreactors or implanted into mice, this synthetic light-control device enabled conversion of a physiologically inert pulsed blue-light beam into a continuous transcription response, the level of which could be adjusted by the irradiation period. Remote control of transgene expression by means of electromagnetic waves may enable quantitative cell culture experiments, providing opportunities for economical manufacturing of difficult-to-express protein therapeutics and for low-risk dosing in gene- and cell-based therapies.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S5
References (*38*–*45*)

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Selective Attention from Voluntary Control of Neurons in Prefrontal Cortex

Robert J. Schafer[1]* and Tirin Moore[1,2]†

Animals can learn to voluntarily control neuronal activity within various brain areas through operant conditioning, but the relevance of that control to cognitive functions is unknown. We found that rhesus monkeys can control the activity of neurons within the frontal eye field (FEF), an oculomotor area of the prefrontal cortex. However, operantly driven FEF activity was primarily associated with selective visual attention, and not oculomotor preparation. Attentional effects were untrained and were observed both behaviorally and neurophysiologically. Furthermore, selective attention correlated with voluntary, but not spontaneous, fluctuations in FEF activity. Our results reveal a specific association of voluntarily driven neuronal activity with "top-down" attention and suggest a basis for the use of neurofeedback training to treat disorders of attention.

Animal and human subjects can learn to alter their own brain activity when they are provided with feedback (*1*–*4*). Voluntary control of neuronal activity is likely associated with changes in behavior or cognitive functions, but that relationship is unclear. Operant control of motor cortical neurons is typically dissociated from movement production (*5*–*8*), and there are no clear behavioral consequences of operant control of neuronal spiking activity in other brain structures (*1*, *4*). Naturally, one might ask whether a chosen control strategy can elicit untrained behavioral or neurophysiological outcomes. To address this question, we examined the consequences of voluntary control of neurons in the frontal eye field (FEF), a visuomotor area within the prefrontal cortex with a known role in the programming of saccadic eye movements (*9*) and visual spatial attention (*10*), in rhesus monkeys (Fig. 1A).

We first asked whether the activity of FEF neurons can be controlled voluntarily by the monkey without explicit training on any task. We used an operant control paradigm (*2*) in which the monkey received juice rewards for alternately increasing and decreasing the firing rates (FRs) of FEF neurons during fixation (*11*) (Fig. 1, B and C). During trials, the monkey received auditory

[1]Department of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305, USA. [2]Howard Hughes Medical Institute, Stanford University School of Medicine, Stanford, CA 94305, USA.

*Present address: McGovern Institute for Brain Research, Massachusetts Institute of Technology, Cambridge, MA 02139, USA.
†To whom correspondence should be addressed. E-mail: tirin@stanford.edu

feedback via pure tones, the pitch of which corresponded to the instantaneous FR of multiunit activity (MUA) at a FEF recording site. In blocks of "Up" trials, the monkey received a reward each time the FR, and thus the tone pitch, reached a high threshold (Fig. 1C). In blocks of "Down" trials, the monkey was rewarded each time a low threshold was reached.

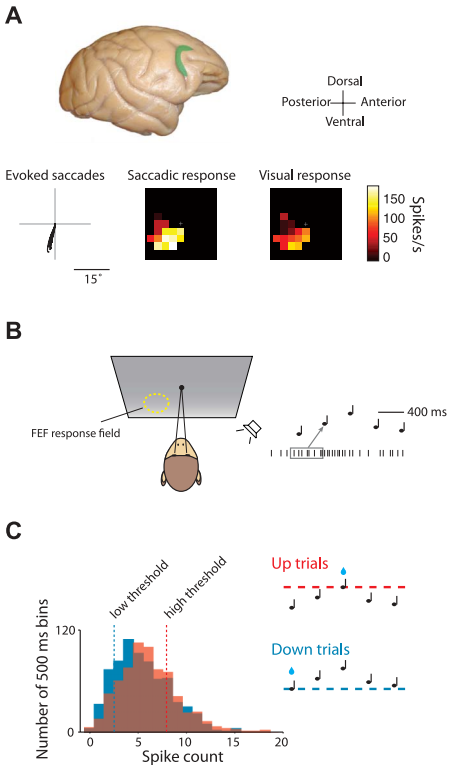


Fig. 1. Operant control paradigm. (**A**) Top: Location of the FEF in the arcuate sulcus (shading) shown in a lateral view of a monkey brain. Lower left: Eye traces show saccades evoked with 50-μA microstimulation of a FEF site. Lower center and right: Saccadic and visual responses, respectively, at the same FEF site during a visually guided delayed-saccade task. (**B**) Operant control task. The monkey fixated a central spot on an otherwise blank video display. Dotted circle shows the FEF response field; speaker icon and musical notes depict auditory feedback of FEF neuronal activity (spike train) during a sliding 500-ms window (open rectangle). (**C**) Spike counts and rewards. Histograms show binned spike counts during Up and Down operant control for the example site in (A). Rewards (blue droplets) were delivered each time a spike count reached the high threshold (red dashed line) on Up trials, or the low threshold (blue dashed line) on Down trials.

We recorded from 94 FEF sites in two monkeys (monkey B, 49; monkey C, 45) during operant control. Figure 2, A to C, shows the results of a representative experiment. At this site, the MUA FR on Up trials was greater than on Down trials (Fig. 2A; $P < 10^{-4}$), indicating that the monkey modulated the MUA in the rewarded direction. This FR difference persisted through the entire trial and was maintained between blocks of Up and Down trials (Fig. 2B). We quantified neuronal control with a "control index" (CI), with positive CIs indicating changes in activity in the rewarded direction. The overall CI for the example site was 0.064, corresponding to a 13.7% increase in FR from Down to Up trials (Fig. 2C).

Across all experiments, monkeys exerted modest but reliable control over the FRs of FEF neurons. The mean CI across sites was 0.031 (Fig. 2D; monkey B, $P = 0.0086$; monkey C, $P = 0.0010$; combined, $P = 10^{-4}$), corresponding to a 6.4% difference in FR. However, the magnitude of control varied considerably throughout the course of each experiment ($P = 0.0045$; fig. S1). Furthermore, for individual FEF sites we found both significant positive and negative effects of control (*11*). Fifty-five experiments (59%) showed individually significant effects of control; of these, 38 (69%) had positive CIs, with a mean of 0.080 ($P < 10^{-4}$), and 17 (31%) had

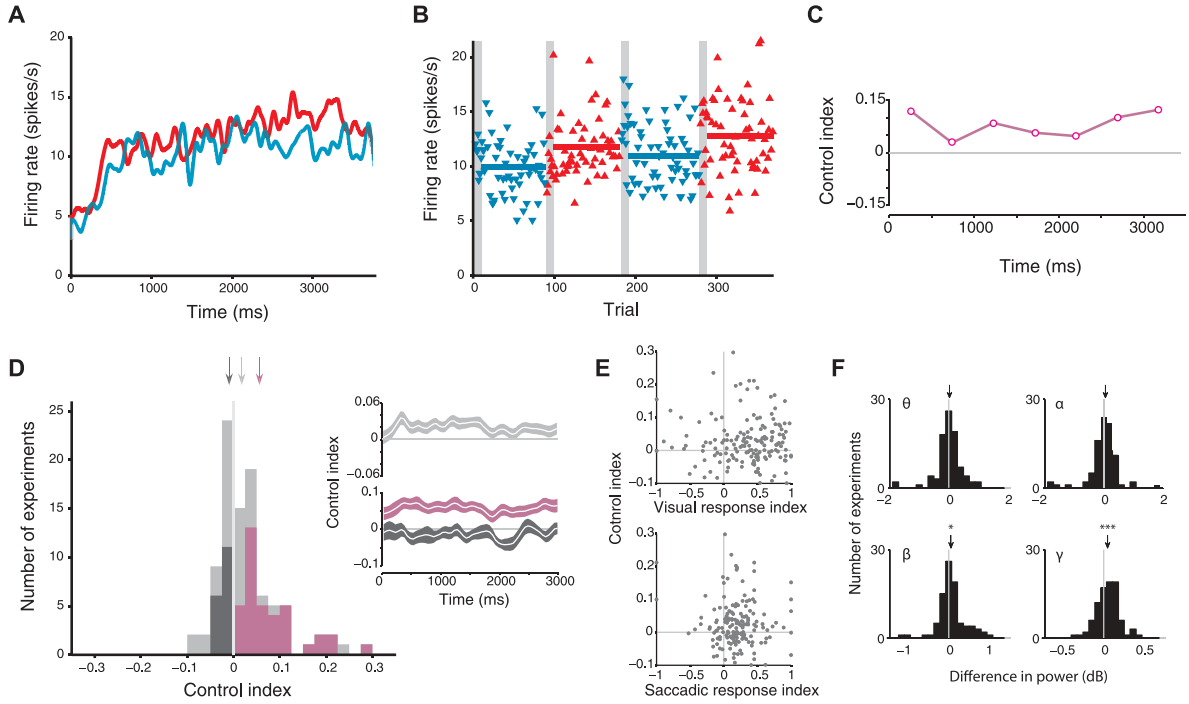


Fig. 2. Voluntary control of FEF neurons. (**A**) Average MUA over the course of Up (red) and Down (blue) trials during an example experiment. (**B**) Firing rates for each Down and Up trial in (A) are indicated by blue and red triangles, respectively, with the mean firing rate for a block of trials represented by a horizontal line. Gray vertical bars mark the first 10 trials after each block transition, which were excluded from analysis. (**C**) Time course of the control index for the experiment in (A). (**D**) Population histogram of multiunit CIs. Light gray histogram shows all experiments; purple histogram shows experiments with individually significant positive control; dark gray histogram shows experiments with significant negative control. Inset shows the mean time course of voluntary control (colors as in the histogram). Thickness of each envelope is ±SEM. (**E**) The control index of single FEF neurons as a function of their visual (left) and saccadic (right) response indices. (**F**) Population histograms showing Up-Down differences in LFP power in four frequency bands. *$P < 0.05$, ***$P < 0.001$.

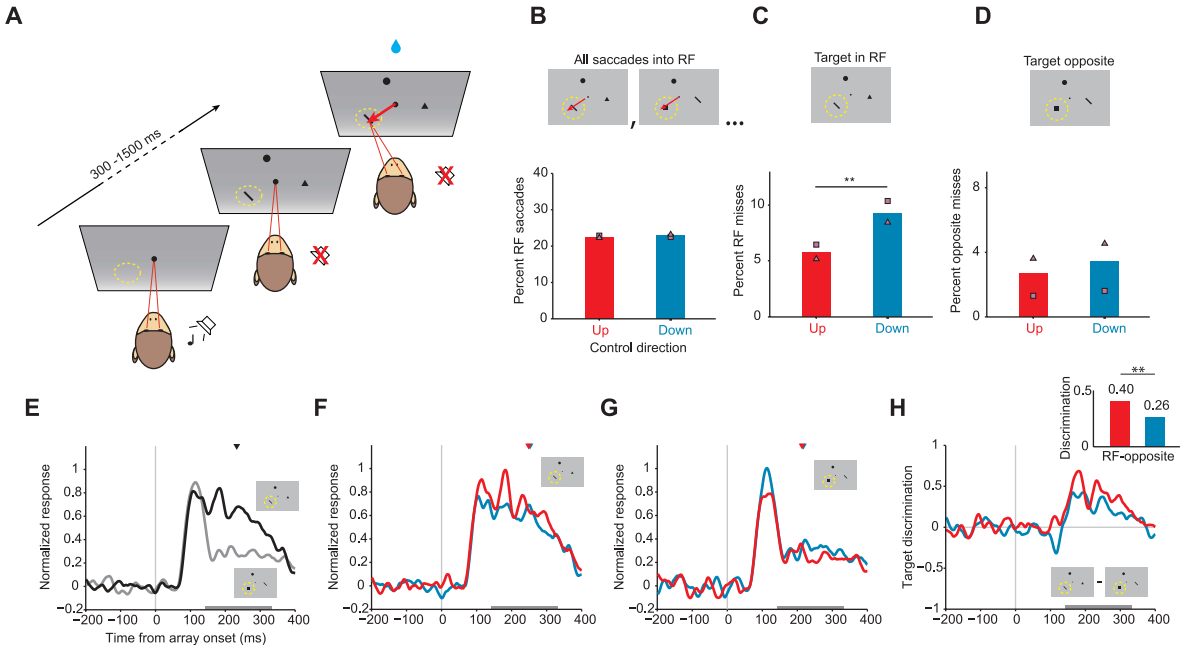
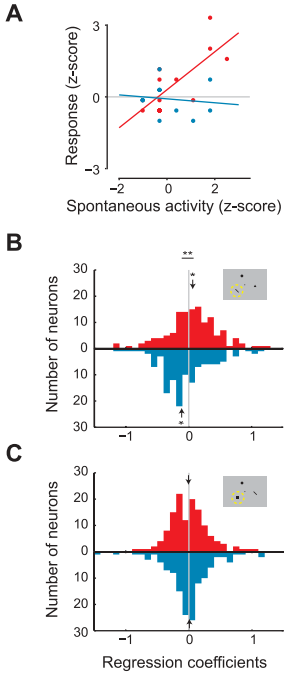


Fig. 3. Behavioral and physiological consequences of operant FEF control. (**A**) Visual search probe trials, in which a search array appeared and the monkey was rewarded (blue droplet) for directing a saccade toward an oriented bar target. (**B**) Percentage of probe trials in which a saccade was directed into the RF, correctly or incorrectly, during all conditions (ellipsis) and during Up (red) and Down (blue) operant control. Purple triangles, monkey B; purple squares, monkey C. (**C**) Proportion of target misses in the RF. (**D**) Proportion of target misses opposite the RF. (**E**) FEF responses to the visual search array. The normalized population response of 150 FEF neurons aligned to array onset for trials with the target in the RF (black) or opposite the RF (gray). Gray bar along abscissa indicates the interval during which the target and distractor responses were significantly different. (**F**) FEF responses to targets in the RF on Up and Down trials. Data are the same as the black line in (E), but red and blue lines show responses on Up and Down trials, respectively. (**G**) FEF responses to distractors in the RF, when the target was opposite. Data are the same as the gray line in (E). (**H**) Target discrimination by FEF neurons, defined as the difference in responses between "target in RF" and "target opposite" trials. Bar plot shows mean target discrimination on Up (red) and Down (blue) trials. $^{**}P < 0.01$.

Fig. 4. Correlation of spontaneous activity with FEF responses. (**A**) Linear regression between the probe trial response of an example FEF neuron and its spontaneous pre-probe activity during Up (red) and Down (blue) control. (**B** and **C**) Population histogram of regression coefficients describing the relationship between spontaneous activity and responses to targets (B) or distractors (C) in the RF. Arrows denote medians of each distribution. $^{*}P < 0.05$, $^{**}P < 0.01$.



negative CIs, with a mean of −0.018 ($P = 0.0002$). The effects observed in MUA were also present in isolated single neurons (*11*). We found no correlation between the CI and the visual ($P = 0.4385$) or motor ($P = 0.1204$) properties of the neurons, indicating that both visual- and movement-related neurons (*12*, *13*) could be operantly controlled (Fig. 2E). The FR effect was also accompanied by a difference in the power spectra of local field potentials (LFPs) at the recording site. LFP power in the beta (13 to 30 Hz) and gamma (30 to 70 Hz) bands increased during Up versus Down trials (β median difference = 0.059 dB, $P = 0.027$; γ = 0.048 dB, $P = 0.00068$), whereas there was no difference for low-frequency theta (4 to 8 Hz) or alpha (8 to 13 Hz) bands (Fig. 2F; θ = 0.033 dB, $P = 0.36$; α = 0.063 dB, $P = 0.33$).

We wondered whether voluntary modulation of FEF activity might have associated effects on behavior or on the function of the controlled neurons. Neuronal control could be achieved through nonspecific means such as changes in general arousal or vigilance, or perhaps through means that were motor in nature but did not require a saccade (figs. S4 to S6). Alternatively, voluntary control might be achieved by a cognitive or behavioral strategy specific to the presumed role of FEF neurons (*10*, *14*). We therefore used visual search trials to probe psychophysical and neuronal performance during operant control. On 29% of operant control trials, the monkey was presented with a visual search array at an unpredictable time (Fig. 3A). Feedback then ceased, and the monkey was no longer rewarded for controlling neuronal activity. Instead, the monkey received a reward for directing a saccade to the search target (an oriented bar) if it was present at any location, or for maintaining fixation if no target was present.

In 82 experiments (monkey B, 41; monkey C, 41), the mean probe trial performance was 86.3% correct (monkey B, 81.9%; monkey C, 90.7%). The overall percentage of saccades targeting the

response field (RF) was the same during Up and Down trials (Fig. 3B; Up = 22.6% of 4441, Down = 23.0% of 4346, $P = 0.65$; monkey B, $P = 0.43$; monkey C, $P = 0.80$). Likewise, the latencies of saccades to RF targets were similar during Up and Down trials (fig. S7; *z*-score normalized Up latency = -0.001 ± 0.033; Down = 0.001 ± 0.032; $P = 0.967$). Thus, we found no evidence that operant control was associated with saccade preparation. The saccadic main sequence was also unaltered by voluntary control (fig. S8).

In contrast, neuronal control had a spatially specific effect on visual search performance. The proportion of trials on which the monkey failed to detect the target in the RF ("misses") was significantly greater during Down trials in both monkeys (Fig. 3C; Up = 5.7% of 1098, Down = 9.3% of 1124, $P = 0.0017$; monkey B, $P = 0.020$; monkey C, $P = 0.032$). In contrast, the proportion of misses at locations opposite the RF was statistically indistinguishable between Up and Down trials (Fig. 3D; Up = 2.7% of 1120, Down = 3.4% of 1114, $P = 0.31$; monkey B, $P = 0.39$; monkey C, $P = 0.70$). The influence of voluntary control on search performance was confined to locations less than ~6° from the controlled neuron's RF (fig. S9). We confirmed that search performance was specifically correlated with the direction of voluntary control and not with spontaneous fluctuations in pre-probe neural activity (fig. S10) (*11*).

Next, we measured the effect of voluntary control on the ability of FEF neurons to identify the search target. As in previous studies (*15*), we found that the responses of FEF neurons (n = 150; monkey B, 61; monkey C, 89) could indicate whether a search target or a distractor appeared within the RF (Fig. 3E) (*11*). However, we also found that the response to RF targets was 16.5% greater on Up than on Down trials (Fig. 3F; Up mean peak-normalized rate = 0.668, Down = 0.573, $P = 0.0054$). In contrast, there was no difference between responses to distractors during Up versus Down trials (Fig. 3G; Up = 0.265, Down = 0.313, $P = 0.9207$). Thus, neuronal control selectively enhanced FEF responses to targets but not to distractors, resulting in a significant enhancement in target discrimination during Up trials relative to Down trials (Fig. 3H; Up = 0.404, Down = 0.259, $P = 0.004$; monkey B, $P = 0.046$; monkey C, $P = 0.037$).

As with the behavior, target discrimination by FEF neurons was specifically related to operant control rather than noncontrolled fluctuations in spontaneous pre-probe activity. Overall pre-probe activity for combined Up and Down trials did not predict the FEF responses to targets (median regression coefficient = 0.0145, $P = 0.9337$). However, dividing trials according to the direction of voluntary control revealed that pre-probe activity was positively correlated with the target response during Up trials (Fig. 4, A and B; median regression coefficient = 0.0633, $P = 0.0303$), but anticorrelated during Down trials (median = -0.1128, $P = 0.0468$; Up versus Down, $P = 0.0033$). In contrast, responses to distractors were uncorrelated with pre-probe activity for both Up (Fig. 4C; median = -0.0072, $P = 0.6560$) and Down trials (median = 1.05×10^{-17}, $P = 0.9853$; Up versus Down, $P = 0.7431$). Thus, pre-probe FR combined constructively with target-driven activity only during upward control.

Our results demonstrate that voluntary control of FEF neuronal activity is associated with spatially selective visual attention, measured behaviorally and neurophysiologically. In controlling FEF activity, monkeys converged on a strategy that dissociated spatial attention from saccade preparation. This untrained dissociation provides a naturalistic demonstration that the two functions are not wholly interdependent—a point that has proven difficult to substantiate (*10*). We also observed that the attentional consequences of operant control were correlated with voluntarily driven, rather than spontaneous, FEF activity. This selective linkage might occur if control alters interactions between the FEF and visual cortex—a possibility supported by the enhanced LFP power at frequencies with suspected involvement in long-range interactions during attention (*16*). Finally, our results suggest a basis for recent evidence that neurofeedback training may be therapeutic in patients with attention deficit–hyperactivity disorder (*17*, *18*), as they demonstrate that voluntary modulation of neural activity can indeed produce specific changes in cognitive performance.

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Supporting Online Material

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Materials and Methods
Figs. S1 to S10
References

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Inducing Sleep by Remote Control Facilitates Memory Consolidation in *Drosophila*

Jeffrey M. Donlea,[1] Matthew S. Thimgan,[1] Yasuko Suzuki,[1] Laura Gottschalk,[1] Paul J. Shaw[1]*

Sleep is believed to play an important role in memory consolidation. We induced sleep on demand by expressing the temperature-gated nonspecific cation channel *Transient receptor potential cation channel* (*UAS-TrpA1*) in neurons, including those with projections to the dorsal fan-shaped body (FB). When the temperature was raised to 31°C, flies entered a quiescent state that meets the criteria for identifying sleep. When sleep was induced for 4 hours after a massed-training protocol for courtship conditioning that is not capable of inducing long-term memory (LTM) by itself, flies develop an LTM. Activating the dorsal FB in the absence of sleep did not result in the formation of LTM after massed training.

Although the functions of sleep remain unknown, sleep is believed to be important for maintaining optimal performance in a large and diverse number of biological processes (*1*, *2*). Historically, the importance of sleep has been most convincingly established by demonstrating negative consequences that accrue in its absence (*3*). In contrast, methods that allow an experimenter to induce sleep on demand are lacking. Thus, it has been difficult to demonstrate that sleep serves a beneficial role per se. Studies in humans indicate sleep may play an active role in the strengthening or stabilizing of new memories (*4*, *5*). With this in mind, we conducted experiments

[1]Department of Anatomy and Neurobiology, Washington University in St. Louis, 660 South Euclid Avenue, St. Louis, MO 63110, USA.

*To whom correspondence should be addressed. E-mail: shawp@pcg.wustl.edu