Carnegie Mellon University

Annual Progress Report: 2012 Formula Grant

Reporting Period

July 1, 2014 – June 30, 2015

Formula Grant Overview

Carnegie Mellon University received $1,028,926 in formula funds for the grant award period January 1, 2013 through December 31, 2016. Accomplishments for the reporting period are described below.

Research Project 1: Project Title and Purpose

Mechanisms of Action Binding in Behavioral and Neural Systems – Many skills involve learning to bind independent actions into a unified sequence of responses. Yet we don't know precisely how the brain performs this type of skill learning, despite ample evidence that certain patient populations (e.g., Parkinson's Disease, Huntington's Disease, etc.) show impairments in procedural skill learning. We propose a research project centered on finding the mechanisms that regulate sequential action binding and determining how this takes place across weeks of training. Understanding how these skills are acquired can provide critical insights into optimal rehabilitation strategies for patients with pathological impairments in skill learning.

Anticipated Duration of Project

1/1/2013 – 12/31/2016

Project Overview

Many real world skills involve learning to bind discrete, independent actions into a unified sequence of responses. For example, every novice piano student understands the frustration of learning to hit the right keys, in the proper order and at just the right time so as to master even a relatively simple melody. This temporal binding of actions typically occurs after weeks of practice where the brain learns to up-regulate the gating of future appropriate responses, and down-regulate unwanted potential actions. Despite extensive research on sequence learning, relatively few studies have focused on how actions get bound together with practice and even fewer have looked at learning across the timescales that everyday skills are acquired (i.e., weeks or months). We propose a research project centered on finding the mechanisms that regulate sequential action binding and determining how this takes place across weeks of training.

We have recently developed a novel metric to quantify response binding in the context of a complex, bimanual sequencing task that is trained over the course of two weeks. Building off of this work, the proposed research project aims to elucidate the computational and neural systems that mediate this binding process. Specifically, we will show how: 1) errors, stimulus-associations, and rewards all influence the ability to bind responses together; 2) the properties of response "chunking" are different after weeks of training compared to just one or two days of practice; 3) this binding is learned by specific sub-systems in the cortico-basal ganglia network; 4) Efficient regulation of reward & inhibitory control pathways are key to learning a complex sequence; 5) individual differences in brain network integrity can predict differences in specific components of the learning process.

Specific Aim 1: Determine the learning mechanism that regulates response binding.
Specific Aim 2: Map the neuroanatomical substrates of response binding.
Specific Aim 3: Determine how individual differences in neural connectivity influence the ability to learn to bind responses together.

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

We anticipate several positive outcomes and benefits from this study. (1) This research program will be the first of its kind to build an integrative, multi-system framework of skill learning across weeks of training. This requires a comprehensive effort that leverages psychophysics, computational modeling, and cutting-edge neuroimaging approaches. The end result will be a set of empirical findings that could not only highlight the root systems that give rise to action binding, but also elucidates the neurobiological sources of individual differences in long-term skill learning. (2) Understanding precisely how action binding is learned is a critical step in developing optimal training regimes for skill learning in neurologically healthy individuals and understanding functional deficits in neurological populations who exhibit impaired sensorimotor learning (Parkinson's disease, stroke, etc.). The findings of this research program can shed light on the fundamental contexts and conditions that facilitate skill learning and be extended to developing optimal educational strategies for classroom environments. In neurological populations, these training strategies can facilitate optimal rehabilitation approaches that take advantage of natural learning dynamics for better recovery outcomes. (3) As a byproduct of this

research program, we will produce the first publicly available database of high-resolution white matter tractography datasets. These datasets can have enormous impact on empirical researchers interested in patterns of anatomical connectivity in healthy populations, as well as neuroscience educators who can use these datasets and the accompanying open source tools to teach basic neuroanatomy.

Summary of Research Completed

Aim 1. In order to distinguish competing mechanisms between two forms of reinforcement learning (Specific Aim 1), we trained subjects to perform a motor sequence learning task that varied the type of feedback participants received after each trial. A total of 40 subjects were randomized to either a reward condition, in which participants received points for successful key presses, or a penalty condition, in which participants lost points from a predetermined sum. Over the course of two days of training, subjects learned to perform a complex motor sequence of discrete key presses. We recorded both the subjects response times and accuracies throughout this training to determine whether or not there was an interaction between the type of reinforcement (reward or penalty) and different forms of learning. Figure 1 summarizes the main results from this experiment.
Although we observed no significant differences in accuracy across the two days of training (Figure 1), the response time learning score showed a significant increase between Day 1 and Day 2 for the penalty group but not the reward group. However, the overall pattern of results was not significant and consensus within the research team was to revise the design of the study for the Fall 2015 recruitment cycle.

Future studies in our lab will explore how the integrity of basal ganglia pathways, thought to be critical in error-corrective reinforcement learning, may mediate the observed behavioral findings. We should also point out that recent work from other labs is highlighting pattern similar to the null results are observing in this experiment.

We also completed analysis and are finishing the manuscript to submit the results of last year's experiment in this aim on different levels of representation of learning. This work was presented at the Society for Neuroscience 2014 conference in Washington DC. The manuscript is expected to be submitted in August 2015.

Aim 2. Little is known about how complex movements are stored in the brain. Since impairments in both the acquisition and execution of complex movements are observed in a large number of neurological disorders including Parkinsons' disease and Huntington's disease, a better understanding of how motor sequences are encoded would be of substantial benefit. In an ongoing experiment, we are training participants to perform a variant of the serial reaction time task, in which subjects learn to perform a complex sequence of key presses across an entire month of training.

A number of studies have shown that as subjects learn complex movement sequences, specific items within that sequence become grouped together as a single unit, a process referred to as chunking (Figure 2). We are examining the networks underlying this process using fMRI to

examine how movements are represented in motor areas, both before training begins, and after training is completed. Using representational similarity analysis on the distributed neural activity associated with each cue-response pairing, we track the distance between the representations of each of the fingers (effectors). This enables us to determine where in the brain the representations of each of the effectors is plastic, thus informing our understanding of what regions are involved in both the generation and acquisition of complex movement sequences.

Furthermore, because the sequence that the subjects learn to perform has been carefully chosen to contain a high frequency of certain key pairings (Figure 3), we predict that certain patches of motor cortex will highly correlate with these representational patterns. Figure 4 shows the surface projections of the distance between effector representations both before (A) and after training (B). Panel A shows that the distance between finger representations encompasses a broad patch of both motor and parietal cortex, while these representations in Panel B are considerably smaller. Future analyses from these experiments will examine in greater detail both cortical and sub-cortical networks that may mediate the observed behavioral changes.

Aim 3. Mastering motor sequences is essential for many daily life activities, such as typing on a keyboard or playing a piano. This form of learning relies on the striatal nuclei that form the principal inputs to a group of subcortical regions known as the basal ganglia. Previous research has also shown that central obesity is associated with compromised striatal functioning, particularly when processing reward signals. Thus, we looked at how physical obesity may be linked to reduced skill learning in a sequential motor task.

Using an indirectly-cued serial reaction time task (SRTT), long-term motor sequence learning was assessed in a cohort of thirty neurologically healthy adults, with body types ranging from lean, to overweight, to obese. Training occurred for five consecutive days in all subjects and principle measures of sequence-specific learning were collected for both accuracy and reaction times.

As expected, individuals with a greater degree of central adiposity, measured as central waist circumference, had slower rates of learning across training days compared to leaner counterparts (Figure 5). This association between learning and central adiposity was restricted to response speeds, but not accuracy. These findings confirm the association between physical obesity and the efficiency of long-term motor sequence learning, suggesting that obesity is a general basal ganglia concern, not just for reward processing, but also motor skill learning. Future work will be undertaken to explore the origin of these group differences at the neural level (Specific Aim 2), by replicating these effects while hemodynamic responses are recorded using fMRI.

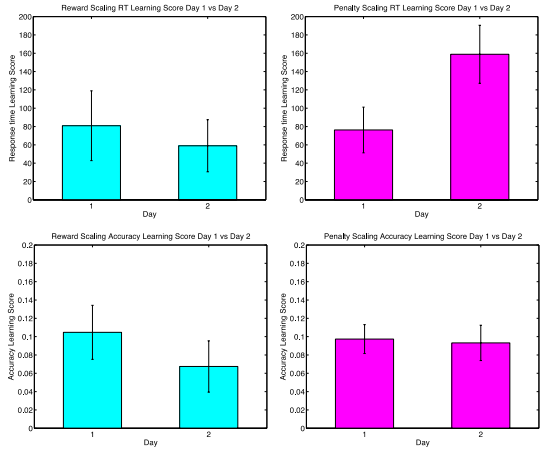

Figure 1. Results of the behavioral experiment described in Section I. Bars show the mean and errorbars show standard deviations.

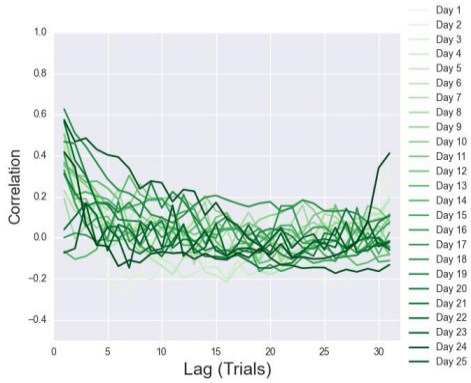

Figure 2. Behavioral evidence of chunking across 25 days of complex movement training measured using an autocorrelation function

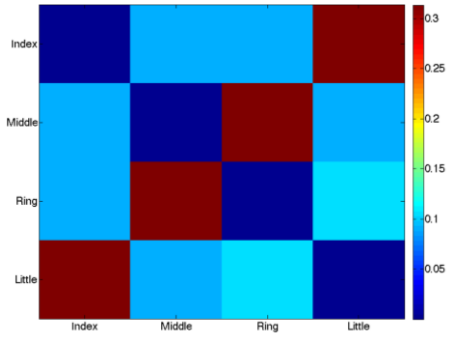

Figure 3. Hypothesized distance between effectors based on the constructed sequence

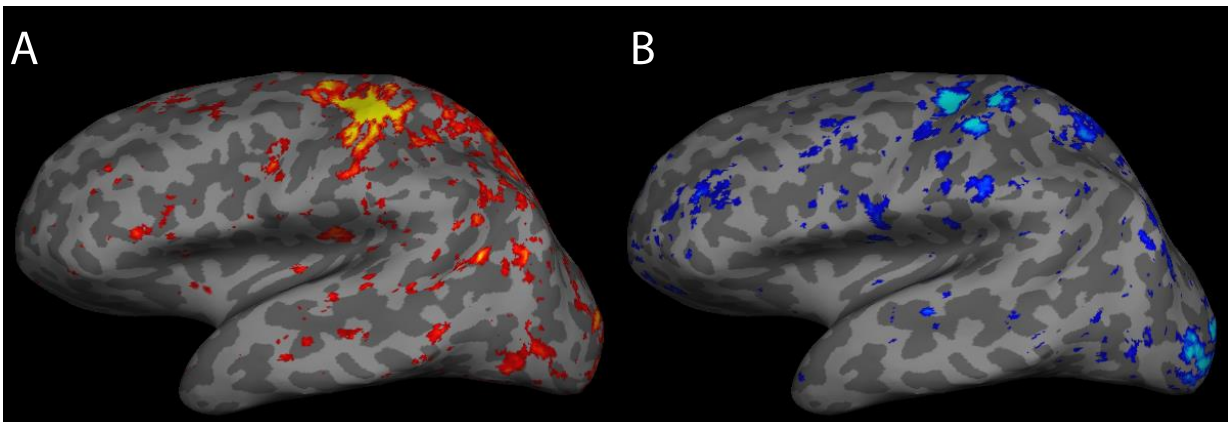

Figure 4 Representational Dissimilarity Analysis: A. Pre (Red-Yellow) and B. Post (Blue-Cyan) Training

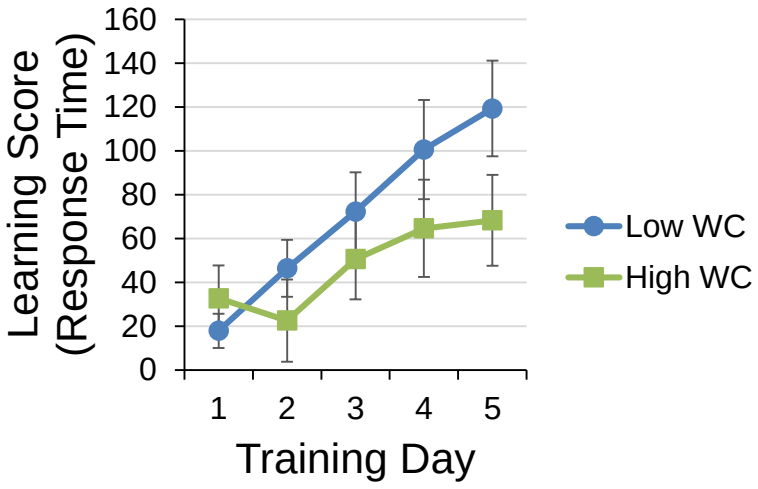

Figure 5: Learning Scores across training days by binary waist circumference (WC) categories, Reaction time learning scores across training days, Error bars based on SEM.

Research Project 2: Project Title and Purpose

Development of Inhibitory Circuits in Visual Cortex – GABAergic inhibition is a key mediator of experience-dependent plasticity during postnatal development, and accumulating evidence identifies aberrant GABAergic function in schizophrenia and autism. Strikingly, there are a number of disease-associated genes that, when mutated specifically in inhibitory neurons, reproduce behavioral deficits that characterize neurodevelopmental disease. However, it is not well understood how dysfunction of signaling pathways in inhibitory neurons impacts cortical function in-vivo. Using in-vivo multi-photon microscopy imaging of mice harboring targeted gene mutations we will evaluate the impact of specific genetic disturbances on circuit function and learning.

Anticipated Duration of Project

1/1/2013 – 12/31/2016

Project Overview

Maturation of the mammalian brain from birth through adolescence is a prolonged process, and represents a period of heightened learning and adaptation of neural circuits to the local environment. However, this period of circuit refinement also represents a heightened vulnerability to genetic defect: adaptive plasticity can become maladaptive in the case of compromised genetic background.

An essential feature of sensory networks is the ability to maintain a stable range of cortical activity despite large fluctuations in sensory input strength, a feature referred to as gain control. In the visual cortex of mice, gain control is mediated by a specific inhibitory cell type, parvalbumin (PV) cells. Deficits in gain control are linked to the neurodevelopmental diseases of autism and schizophrenia. Our working hypothesis is that deficits in the postnatal development of gain control have negative impact on circuit function and compromise future learning. A defining property of PV cells (compared to other inhibitory cells) is their non-selective response properties; for example these cells in visual cortex are broadly tuned to orientation. We hypothesize that broad tuning of PV cell responses is essential for this cell type to perform the role of gain control, and that broadening is mediated specifically by the development of local recurrent feedback from differentially tuned excitatory neurons within cortical layer 2/3. The following aims are motivated by our recent finding that broad tuning of PV cells develops postnatally, and requires sensory experience; and that synapse formation onto PV cells require tyrosine kinase (ErbB4) signaling. Notably, genetic disturbance of ErbB4 signaling is associated with poor gain control in schizophrenia.

Aim 1: Identify the circuit elements responsible for the development of broad tuning in PV cells.
Experiment 1a. Determine whether developmental broadening is mediated by increased synaptic drive versus an upwards shift of tuning curve. 30 cells from eight animals will be recorded from in each treatment condition.
Experiment 1b. Repeat Experiment 1a in the presence of NMDA receptor antagonists administered acutely during the recording experiment to establish that it is signaling via AMPA receptors that mediates developmental broadening. 30 cells from eight animals will be recorded from in each treatment condition.
Experiment 2. To further establish that it is a maturation of excitatory drive onto PV cells (versus decreased/ or altered inhibitory currents), we will perform in-vivo voltage-clamp whole-cell recordings to isolate excitatory glutamatergic current from inhibitory current (same protocol as Kuhlman 2010 used in-vitro), also in the anesthetized prep. 20-30 cells from eight animals will be recorded from in each treatment condition.
Experiment 3. Determine whether recurrent L2/3 connectivity from excitatory neurons onto PV neurons increases with age.

Aim 2: Characterize the contribution of ErbB4 signaling to the broadening of PV cell responses.
Experiment 1a. Administer neuregulin ligand (intraventricular injection) to mice at age P17-19 and assay orientation tuning of PV neurons using in-vivo 2-photon guided targeted.
Experiment 1b. Following neuregulin administration we will assay connectivity probability in-vitro as in Aim1 Experiment 3.
Experiment 2. Down regulate neuregulin/ErbB4 signaling by administering a widely used antagonist of ErbB4 signaling, EctoErbB4.
Experiment 3. Although in cortex ErbB4 receptor is expressed only in inhibitory neurons and is highly enriched in the PV subtype, it is also expressed in other inhibitory subtypes. Therefore to convincingly establish that the above expected results are due to signaling within PV neurons, we will specifically manipulate ErbB4 signaling in PV neurons by using the cre/lox strategy and

crossing PV-cre mice with heterozygous floxedErbB4 mice to specifically knock down ErbB4 receptors only in PV cells.
Experiment 4. To further establish that neuregulin is acting at ErbB4 receptors expressed specifically in PV cells, we will determine whether the accelerated development of broad orientation tuning is occluded in mice lacking ErbB4 receptor expression.

Aim 3: Evaluate the impact of functional broadening of PV cells on (1) wiring-up of top-down, feedback inputs from other brain areas onto PV cells, and (2) new skill acquisition.
Experiment 1. The presence of topographically organized feedback originating from secondary cortex and terminating in primary sensory cortex will be functionally assayed by constructing and aligning two maps, one of primary cortex retinotopy and one of secondary cortex axonal activity within primary cortex.
Experiment2a. To establish whether activity of top-down, feedback inputs terminating in primary visual cortex are required for successful perceptual learning we will a three-object bisection task, modified for mice in which 2 flanking vertical lines or poles are presented along with a third vertical line or pole appearing in between the two flankers.
Experiment2b. To next evaluate whether top-down mediated recruitment of global inhibition within primary cortex is required in the three-object bisection task we will compare the performance of control mice to mice in which broad tuning of PV cell responses was blocked with EctoErbB4.

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Other Participating Researchers

None

Expected Research Outcomes and Benefits

We anticipate several potential outcomes and benefits resulting from this study. (1) A devastating impact of neurodevelopmental diseases such as autism and schizophrenia is the failure of afflicted children to reach developmental milestones and ultimately achieve independence. Knowledge of how cell-type specific deficits hinder new skill acquisition and learning will facilitate the rational design of behavioral and drug therapy. (2) Graduate students supported by this grant will be extensively cross-trained in imaging, electrophysiological, molecular, and behavioral approaches, placing them in a unique position to simultaneously conduct hypothesis-driven research and at the same time be open to discovering the unexpected through careful observation of sensory circuits as mice learn new skills. (3) Currently, pharmaceutical companies are developing ErbB4-targetting small molecules for the treatment of

breast cancer. The experimental techniques developed here will provide a means by which we can explore additional therapeutic uses for these drugs. Specifically, the in-vivo tuning assay of PV cell responses can be used in conjunction with mice harboring the mutated genes associated with schizophrenia or autism, to screen these ErbB4-targetting small molecules and assess their potential to alleviate problems of neural gain control.

Summary of Research Completed

Our goals for the current reporting period were to identify the source of broad tuning in PV cells, and determine whether maturation of top-down, feedback into from V2 on neurons in V1 is delayed relative to feedforward connectivity onto these neurons. These goals are described in Aim1 and experiment 1 in Aim 3.

To achieve the research milestone of Aim1, we experimentally tested the hypothesis that the source of broad tuning in the mature circuit is likely derived locally from increased synaptic input originating from differentially tuned excitatory neurons within cortical layer 2/3. In vivo whole cell-recordings of PV neurons were performed (Figure 1) and we discovered that subthreshold synaptic input onto PV neurons broadens with age (adult [postnatal (P) day age 27]: n= 30 neurons, 15 animals; 30/30 neurons were well fit by a 2-peak Gaussian function and had R values >0.8; in contrast, young [P19]: n= 31 neurons in 16 animals; 5/31 neurons were well fit by a 2-peak Gaussian function and had R values >0.8). Mice open their eyes at P13, thus these age groups represent mice approximately 1 week after eye opening, and 2 weeks after eye opening. Voltage clamp recording were performed in the same cells to isolate glutamatergic current from inhibitory current. We found that the excitatory current was described by the same 2-peak Gaussian curves and did not detect a difference in inhibitory current tuning curves. Additionally, in a separate set of animals (n= 4 neurons, 3 animals) we found that hyperpolarizing the recorded neuron did not alter tuning in the adult (Figure 1). These results rule out the alternative to the above synapse development model: the appearance developmental broadening is not due to an 'iceberg' effect of simply raising the tuning curve of suprathreshold spike responses due to increased intrinsic excitability of PV neurons. To provide further evidence that the above results are mediated by AMPA receptors, the above experiments in P27-aged mice were repeated in the presence of NMDA receptor antagonists administered acutely during the recording experiment (n=23 neurons in 12 animals) and the resulting orientation tuning curves were not different from those without NMDA antagonists (23/23 neurons were well fit by a 2-peak Gaussian function and had R values >0.8). Taken together, these results suggest that it is signaling via AMPA receptors that mediate developmental broadening of orientation tuning curves.

We also performed in vitro slice electrophysiology experiments in which paired recording was performed (Figure 2). We found that the connection probability between pairs of synaptically connected neurons (Ex to PV) increased from young (P19, n=30 pairs tested, 13 were connected) to older (P27, n=31 pairs tested, 19 were connected). These results support the primary hypothesis of Aim 1, that developmental broadening is mediated by increased excitatory synaptic drive onto PV neurons.

To achieve the research milestone of experiment 1 in Aim3 it was necessary to develop new technology and methods. First, we describe the method that we developed during this reporting period, then we describe the results of the experiment performed using the newly developed method. The presence of topographically organized feedback originating from secondary cortex (V2) and terminating in primary sensory cortex (V1) was functionally assayed by specifically silencing higher visual area 'LM' (lateral medial area) that is most homologous to V2 in the primate. Adeno-associated virus (AAV) was used to transfect visual cortex with channelrhodopsin and specifically LM was silenced (see experimental details below). We confirmed that this method was both (1) effective in silencing LM, and (2) spatially restricted to LM (Figure 3). Next we used intrinsic signal optical imaging (ISI) to retinotopically align the area of LM silencing with the area of V1 in which the neurons were recorded (Figure 4). ISI is a standard technique to identify the location of V1 and to define the border between V1 and V2. It was necessary to define the boarder so that we could ensure that photo-inhibition was specific to LM and did not occur over V1.

We examined the role of LM in development by silencing LM and determining the effect of silencing on visually evoked response properties in three different age groups of mice (P19, P27, and P45) and used two treatment conditions. Control treatment, and mice treated with neuregulin to accelerate maturation of PV neurons. We found that in the P45-control treatment group LM feedback facilaites visual evoked responses by 22% (Figure 5; n=16 neurons, 12 animals). Neuregulin treatment had no impact on the effect of LM silencing. In contrast, LM silencing did not alter visual responses at younger ages (P 19, LEDoff: 1.9±0.3 Hz, LEDon: 2.0±0.2 Hz; P27 LEDoff: 2.1±0.2 Hz, LEDon: 2.2±0.3 Hz). These results indicate that the development of LM feedback to V1 is delayed compared to development of feedforward inputs to V1.

Experimental details:

In-vivo visual stimulation and recording Full-field drifting square-wave gratings are presented at varying contrasts, at 6 orientations spaced 30 degrees apart at two directions of motion (12 orientations). Varying spatial frequencies (0.02 and 0.04 cycles/°) and temporal frequencies (1-2 Hz) are tested and the stimulus giving the best response is shown for 4-9 trials. Whole-cell internal solution: 115 mM potassium gluconate, 20 mM KCl, 10 mM HEPES, 10 mM phosphocreatine, 14 mM ATP-Mg, 0.3 mM GTP, and 0.01–0.05 mM Alexa-488.

In-vitro slice electrophysiology: Unitary excitatory and inhibitory post synaptic currents (uEPSCs and uIPSCs) were recorded by submerging brain slices in ACSF (in mM): 126 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1 $MgSO_4$, 2 $CaCl_2$, 10 D(-)-glucose, and 25 $NaCHO_3$ continuously bubbled with 95%O_2/5%CO_2, perfused at a rate of 2-3 ml/min (33±1°C). Signal were digitized using NiDaq acquisition board and WinEDR software, samples at 10 kHz.

In-vivo photo-inhibition The location of the electrophysiological recording site in primary visual cortex was retinotopically matched to the location of silencing in the lateromedial higher visual area (LM) as determined by the intrinsic signal imaging maps for each animal (Figure 3). In order to silence LM, we stimulated local PV-positive neurons using a light activated depolarizing

ion channel, channelrhodopsin. Briefly, we modified the two-photon microscope by adding a dichroic mirror (485 LP, Omega Optical) before the viewing head, allowing the illumination of the mouse cortex through a 40x objective with a 470nm LED light (Mightex). To restrict the size of the LED illumination, a 4mm pinhole was introduced in the path of the LED light. Due to the heterogeneity in the levels of channelrhodopsin expression between animals, at the beginning of each experiment, the intensity of the LED light was calibrated by recording visually responsive Ex neurons in LM and assessing the effect of different LED intensities on their response. The minimal LED intensity that produced 100% silencing was used for the experiment. The average intensity was 99 ± 21 μW. To define the spatial limits of silencing, we recorded LED evoked spikes from PV neurons centered directly under the objective and then systematically translated the objective to various distances from the recorded neuron. Using this approach, we found that the radius of silencing is 350 microns.

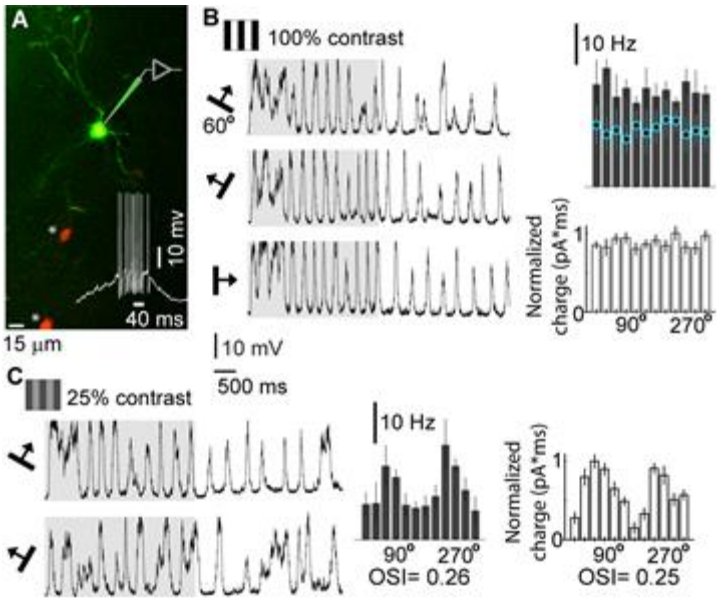

Figure 1. ***In vivo whole-cell recording of an example PV inhibitory neuron.***
A, 2-photon image, red and green channels are merged. Recording electrode was filled with green-emitting Alexa-488 dye as indicated by schematic. Two PV neurons not recorded from (asterisks) appear in same filed of view as the recorded neuron (yellow indicates merged green and red fluorescence). Note the processes of recorded neuron are filled with Alexa-488. Inset, spontaneous spikes.
B, example traces of synaptic potentials in response to drifting grating at 100% contrast (gray shading, 3 sec in duration), action potentials clipped. Evoked spike rate tuning curve (dark bars ± Std, OSI= 0.02) and synaptic potential tuning curve (open bars ± SEM, 1st cycle, OSI=0.01) shown far left, Vrest 70 mV. An evoked spike tuning curve was acquired while Vrest was set to -85 mV with slow current injection in current clamp mode (blue points, OSI=0.02).
C, traces and plots as in B, except in response to 25% contrast. Note the increased synaptic potential at the preferred orientation (60 deg.), compared to non-preferred orientation.

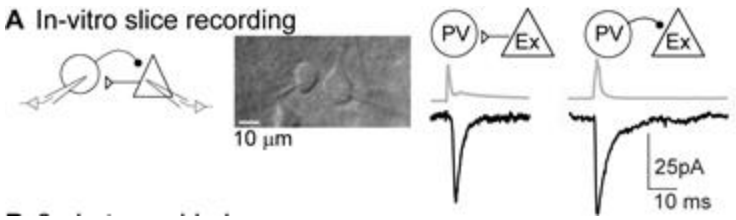

Figure 2. ***In vitro slice 'paired' recording.***
Schematic of recording configuration shown far left. Center, nIR-DIC image of cortical slice with two recording electrodes. Right, pre-synaptic stimulation shown in gray, unitary post-synaptic responses are bold; leftmost is a Ex-to-PV synaptic connection, rightmost is a PV-to-Ex connection.

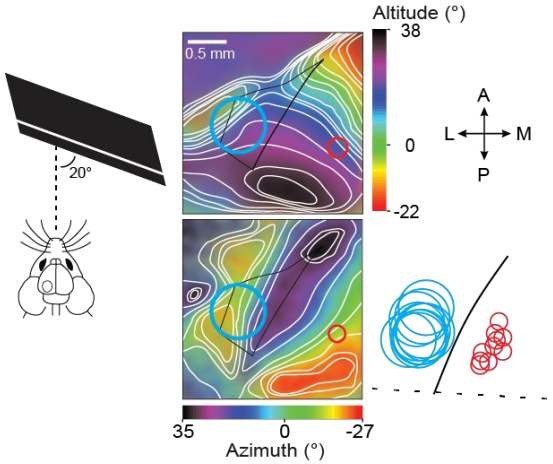

Figure 3. ***Intrinsic signal optical imaging of visual cortical areas V1 and LM.***
Left, schematic of mouse relative to the angle of the monitor used to activate contralateral visual cortex. Center, cortical representation of visual space is indicated in pseudocolor as indicated by scale bars, in degrees for one example animal. Right, area of photo-inhibition (blue circles) and recording sites (red circles) projected onto an average space map of all animals used (n=9). Black line indicates boarder between primary visual cortex (V1) and lateral medial area (LM). Scale bar: 500 microns.

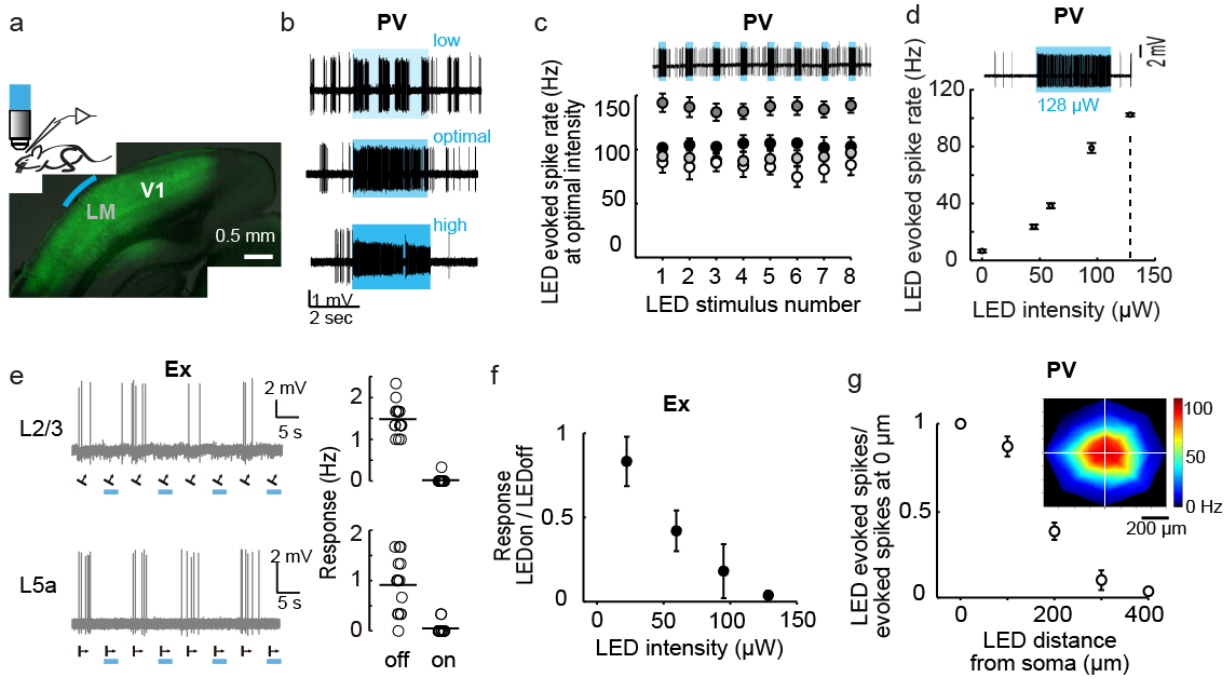

Figure 4. Novel method to spatially restrict photo-inhibition of visual cortex
(a) Epi-fluorescent image of PV neurons expressing AAV (green) in a coronal brain slice that includes LM and V1. (b) Activation of PV neurons depends on LED intensity. (c) When using optimal LED intensity, light-induced firing of PV neurons is repeatable and reliable. (d-f) Example animal that confirms LED intensity calibration activates PV neurons in optimal range [d], and reliably silences excitatory (Ex) neuron action potential generation [e-f]. (g) PV neuron light-evoked firing rate is highest at the center of illumination, and is restricted to a 350 micron radius. Inset is an example neuron, the plot is an average of 4 neurons from 3 different animals.

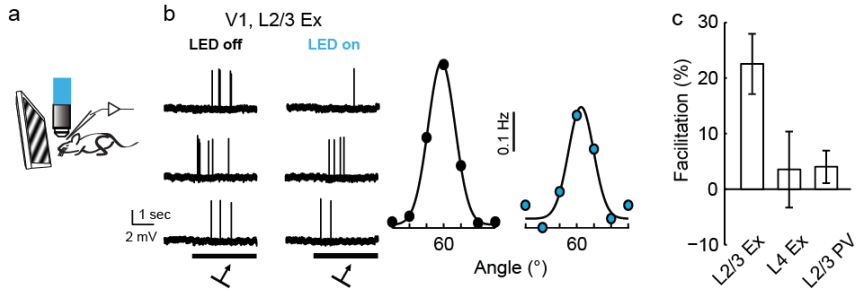

Figure 5. *Quantification of LM feedback on V1*
(a) Schematic of experimental set-up in which mouse is positioned in front of monitor. (b) Example V1 neuron response to visual stimulation with and without LM silencing. (c) summary of all cells recorded in V1.