Carnegie Mellon University

Annual Progress Report: 2012 Formula Grant

Reporting Period

July 1, 2013 – June 30, 2014

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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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

The originally proposed milestone(s) for 7/1/2013-6/30/2014 were to recruit a postdoc, run Experiment 1, start Experiment 2 and begin the computational modeling component.
All work performed was consistent with the originally proposed scope, though we have somewhat modified our experimental protocols in keeping with the most contemporary findings in the neuroscience community.

Experiments 1 & 2 are designed to address Specific Aim 1.

Experiment 1: Sequence complexity and response binding. The associative learning model predicts that actions get bound together with increased frequency of association. Experiment 1 is designed to evaluate this by testing the following hypothesis:

> Hypothesis 1.1: More frequently paired responses will have a greater degree of correlation than less frequently paired responses.

Inclusion of the MFP block will also allow for the evaluation of a secondary hypothesis.

> Hypothesis 1.2: Response binding is learned in an internal, hand-based coordinate frame rather than an external, cue-based coordinate frame.

Based on new findings in the literature (e.g., Acuna et al. J. Neurophysiology 2014), we determined that we should modify the design of Experiment 1. The protocol revisions are:

- Revising the cue interface to be non-spatially indirect cuing (in order to maximize explicit learning).
- Using a remapping procedure to dissociate direct from indirect sequence learning. Thus the first experiment is split into 2 groups: cue learning and response learning. This increases the efficacy of interpreting *where* these complex response bindings occur. In addition we now include a third control group that learns both the cue and the motoric sequence.
- Reducing the number of training days from 10 to 5 (pilot work showed that subject retention was very low at the longer training schedule).
- With the reduction of training days, the sequence cuing was changed to being unimanual in order to speed up learning.
- Using a shorter 12-item cue sequence.

Using an indirect cuing version of the serial reaction time (SRT) task, we measured the independence of cue and response learning across a 5-day training period. On each trial, a centrally presented symbol (Cyrillic letters) cued subjects to press one of 4 keys on a keyboard with their right hand. On each day, the mapping from cue to key was pseudo-randomly assigned and subjects were trained to learn this new mapping. After two blocks (144 trials per block) of randomly ordered cues, subjects were trained on a hidden 12 item sequence for two blocks, followed by another random block and then a final sequence block. Subjects were randomly

placed into two groups (N=15, 6 males per group). The Cue group was exposed to the same sequence of visual cues over all 5 training days. The Response group was exposed to different orders of visual cues but repeated the same sequence of key presses across days.

As shown in Figure 1, learning-related changes in response time showed a significant group-by-day interaction ($F(4,112)=3.72$, $p = 0.007$). Response times during sequence blocks, relative to random blocks, reached asymptote in the Cue group by Day 3, but no such asymptote was present in the Response group. Accuracy improved over time (main effect: $F(4,112)=9.06$, $p<0.001$) but both groups learned to improve their accuracy similarly across days (group-by-day interaction: $F(4,112)=2.30$, $p=0.064$). This advantage for learning consecutive cues did not appear to relate to knowledge of the sequence since both groups showed similar levels of explicit awareness, based on a post-hoc questionnaire. These results show that there is an immediate advantage to learning sequences of visual cues over sequences of actions during long-term skill training.

Experiment 2: Reinforcement context and response chunking. Another way to distinguish associative from error-corrective models is to look at how the reinforcement context modulates learning. Experiment 2 is designed to test the following hypotheses:

> Hypothesis 2.1: Increased reward for successful responses will increase response prediction and state memory during learning, but not error-corrective processes.
> Hypothesis 2.2: Increased penalty for errors will increase error-corrective processes, but not predictive or state memory responses.

We are slightly behind schedule in starting Experiment 2. We did purchase and set up an electromyography device that will be utilized for it.

Experiment 3 (designed to address Specific Aims 2 & 3): Learning-related changes in basal ganglia pathways. While animal studies have implicated the basal ganglia pathways in response binding, it is not known what part of this distributed circuit mediates this learning. Knowing which sub-system in the basal ganglia network is associated with response binding will provide critical insights into the underlying computations that are involved. With this in mind, Experiment 3 is designed to address the following hypothesis:

> Hypothesis 3.1: Learning to bind responses together with long-term training will modulate both the task-evoked dynamics of the basal ganglia and the functional connectivity between the striatum and cortex.

A second aim of Exp. 3 is determining how differences in the integrity of network connections can explain differences in learning abilities. To this end, the subject sample will be carefully designed to maximize variation along dimensions that have been shown to relate to white matter integrity: age, smoking, and body type. This will maximize variation in white matter integrity in non-clinical populations in order to address a second set of hypotheses:

> Hypothesis 3.2: Variation in the anatomical integrity of cortico-basal ganglia connections correlates with variation in functional dynamics of the system.
> Hypothesis 3.3: The integrity of specific segments of the cortico-basal ganglia network can predict individual differences in learning to bind responses together.

Experiment 3 requires finishing Experiment 1, which we are doing now. However, we have

done *pilot work* to show proof of concept that it is possible to identify topographic structure in white matter pathways. These two pilot experiments are necessary in order to meet the goals of Aim 3 and construct the appropriate analysis of Experiment 3.

The first pilot study was designed to confirm that individual differences in connectivity relate to learning. Accurately making a decision in the face of incongruent options increases the efficiency of making similar congruency decisions in the future. Contextual factors like reward can modulate this adaptive process, suggesting that networks associated with monitoring previous success and failure outcomes might contribute to this form of behavioral updating. To evaluate this possibility, a group of healthy adults (N=30) were tested using functional MRI (fMRI) while they performed a color-word Stroop task (see Figure 2A-2C). In a conflict-related region of the medial orbitofrontal cortex (mOFC), stronger BOLD responses predicted faster response times (RTs) on the next trial. More importantly, the degree of behavioral adaptation on RTs was correlated with the magnitude of mOFC-RT associations on the previous trial, but only after accounting for network-level interactions with prefrontal and striatal regions. This suggests that congruency sequencing effects may rely on interactions between distributed corticostriatal circuits. This possibility was evaluated by measuring the convergence of white matter projections from frontal areas into the striatum using diffusion weighted imaging. (Figure 2D). In these pathways, greater convergence of corticostriatal projections correlated with stronger functional mOFC-RT associations that, in turn, provided an indirect pathway that linked anatomical structure to behavior. Thus, distributed corticostriatal processing may mediate the orbitofrontal cortex's influence on behavioral updating, even in the absence of explicit rewards.

The second pilot explored whether there are unique striatal regions that exhibit convergent anatomical connections from orbitofrontal cortex (OFC), dorsolateral prefrontal cortex (DLPFC), and posterior parietal cortex. Deterministic fiber tractography on diffusion spectrum imaging data from neurologically healthy adults (N=60) was used to map fronto- and parieto-striatal projections. In general, projections from cortex were organized in a rostral-caudal gradient along the striatal nuclei; however, we also identified two bilateral convergence zones—one in the caudate nucleus and another in the putamen—that consisted of voxels with projections from OFC, DLPFC, and parietal regions. The distributed cortical connectivity of these striatal convergence zones was confirmed with follow-up functional connectivity analysis from resting state fMRI data from 55 of the participants, in which a high percentage (62-80%) of structurally connected voxels also showed significant functional connectivity. These results delineate a neurologically plausible network of converging corticostriatal projections that may support the integration of reward, executive control, and spatial attention that occurs during spatial reinforcement learning.

<u>Additional work on Specific Aim 1</u>:
In order to build the computational model proposed as part of Aim 1 we have implemented a modified drift diffusion model that can fit the parameters for motoric representations and action selection representations. This required collecting data in a pilot experiment, which we have completed.

We tested the behavioral and neural separability of two types of inhibitory control using a modified stop-signal task. Subjects were instructed to stop a rising bar when it intersected a

target line (500ms after onset) by pressing a key. Subjects were instructed not to respond if the bar did not intersect the line. In Reactive stopping trials, the bar would stop at various intervals in its trajectory. In Proactive stopping trials, the bar would stop 50ms before intersecting the line and subjects were told to make a go/no-go decision based on a color cue indicating the probability that the bar would stop on any given trial. Behaviorally (N=61, 28 male) we found a weak correlation between stopping performance in Reactive and Proactive tasks ($r=0.24$, $p=0.03$, r-square = 0.06) suggesting that the ability plan a go/no-go decision is only weakly coupled with ability to suppress an unwanted action. In addition, performance in the Reactive task was modulated by reward contingencies, while Proactive task performance was not. Finally, event-related fMRI analysis (N=28, 7 male) showed that successful Proactive stopping differentially engaged rostral prefrontal areas in the superior frontal gyrus and anterior cingulate, while Reactive stopping engaged more caudal premotor and pre-SMA regions. From this behavioral and neural evidence, we propose a contingent two-stage decision model of behavioral inhibition. The model frames go/no-go decisions as a competitive drift-diffusion process in which the rate of evidence accumulation towards the go boundary is modulated by contextual factors (e.g., probability or reward). In the event of a stop-signal, a second process is initiated in which a strong inhibitory signal must override the current level of evidence in order to suppress the response. This nested decision model provides a plausible framework for how different executive processes interact during inhibitory control.

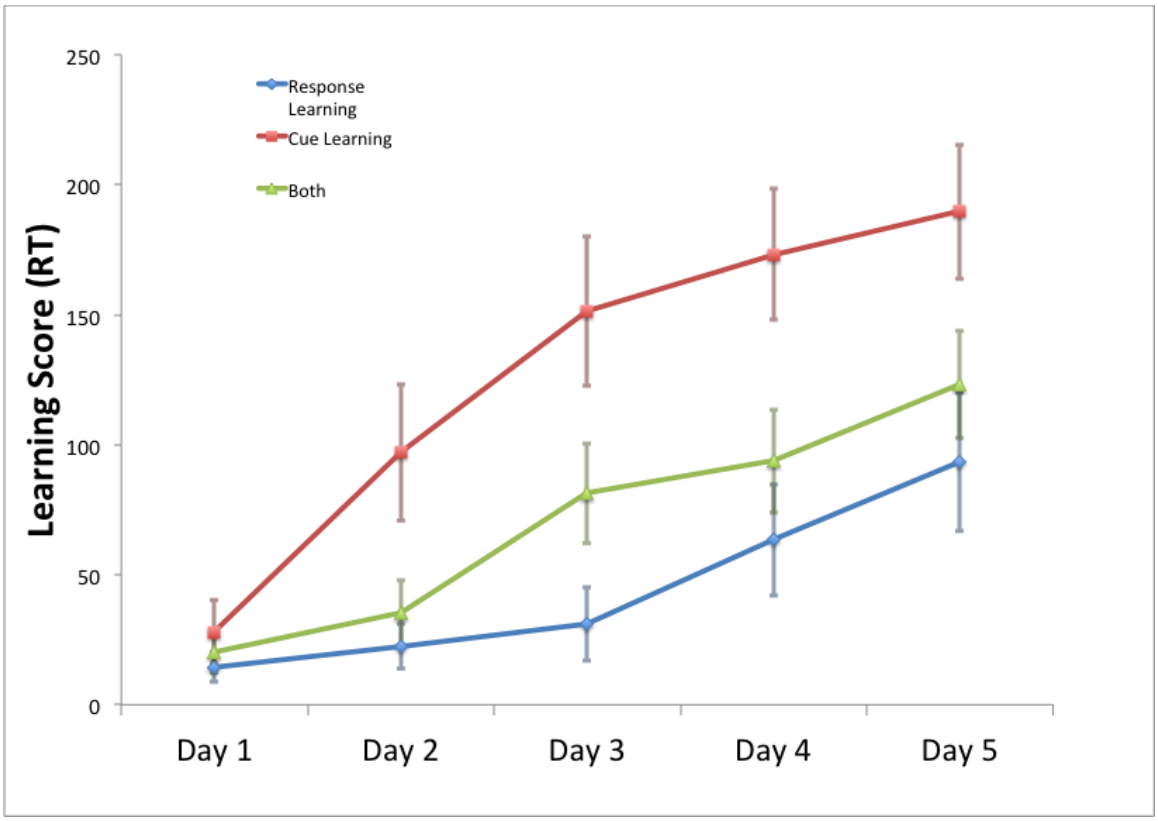

Figure 1: Learning scores for response times on the sequence tasks for Experiment 1. The Cue learning group (red lines) acquired the sequence at nearly 2x the rate of the motoric learning group (blue lines)

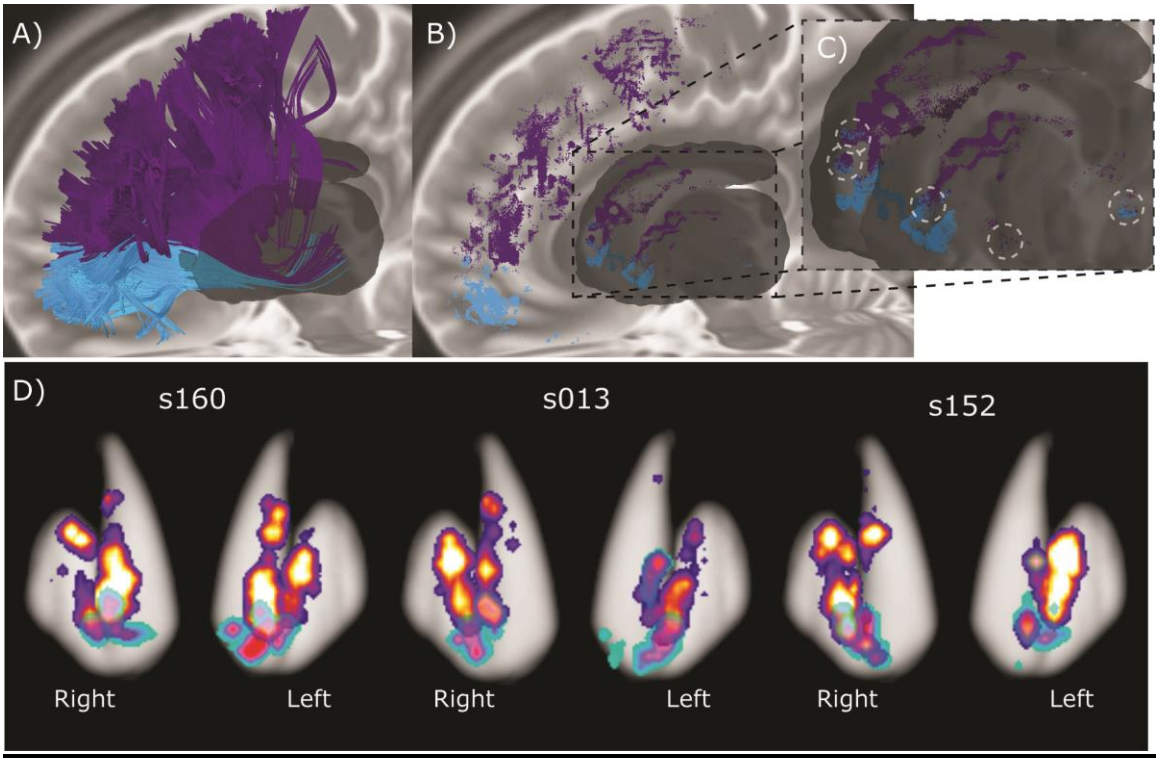

Figure 2. Quantification of overlapping projections in the corticostriatal pathways. This is a proposed mechanism for how information across representation frames gets integrated during learning (goals of Exp. 3)

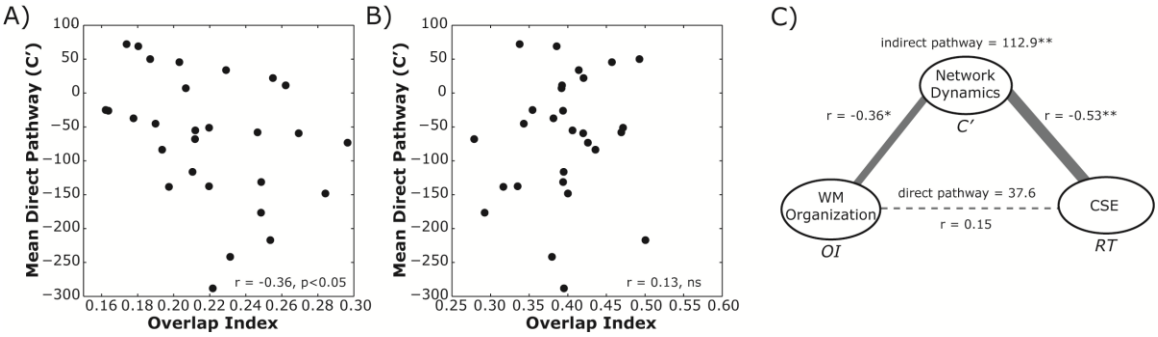

Figure 3 Evidence that the organization of corticostriatal pathways facilitates rapid learning. We are extending this experiment to long term learning of consolidated motor sequences as described in Experiment 3.

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 research goal for the period of July 1, 2013 - June 30, 2014 was to complete Aim2, experiments 1-2, 4: Determine whether broadening of PV cell tuning response can be accelerated by prematurely enhancing ErbB4 receptor signaling. The overall working hypothesis is that neuregulin, realized by excitatory neurons, stabilized excitatory synaptic input onto PV neurons via phosphorylating ErbB4 tyrosine kinase receptor expressed in PV neurons. The phosphorylated receptor then recruits the PSD-95 scaffolding factor to stabilize AMPA receptors at the postsynaptic surface of PV neurons.

Specifically, our goal for this last year was to determine whether the ErbB4 ligand, neuregulin, is sufficient to accelerate the development of PV neurons. To achieve this goal, we injected neuregulin in to the ventricles at age postnatal day P17-19. We confirmed the procedure in two ways:
(1) Correct targeting of injection was confirmed by DiI/ cresyl violet injection (Figure 1b, upper panel). Animals were perfused with phosphaste buffered saline followed by 4%

paraformaldehyde and brains were sectioned at a 60 micron thickness following the injection. Brains were mounted onto microscope slides and coversliped in mounting medium. Injection site was visualized under epifluorescence microscopy using a far-red filter set in conjunction with a arc-lamp light source and a 10x microscope objective. Lateral boarders of ventricles in the tissue sections were identified, as indicated by dark lines in Figure 1b, left.

(2) Western blotting techniques we used to confirm that the injections caused an increase in ErbB4 phosphorylation. Cortical brain extracts were prepared from control and treated conditions. Note that the intensity of the ErbB4-P band in the western blot (Figure1c) is higher in the treated condition compared to the control condition. Staining for actin was included to demonstrate that a similar level of protein extracted was loaded into the two lanes shown.

Results

Experiment 1a

Our in-vivo recordings of PV neuron activity revealed that development of PV broadening and spike rate was accelerated by NRG injections (Figure 1).

Specifically we found that PV neurons are more broadly tuned (OSI decreased by 50%) in animals injected with NRG (n=5 neurons, OSI: 0.09± 0.02) compared to age-matched controls (OSI: 0.18 ± 0.01).

Experiment 1b

Our in vitro recordings of connection probability revealed that there was a slight, but not statistically significant increase in connection probability between layer 2/3 glutamatergic excitatory neurons and L2/3 PV neurons in response to neuregulin administration (probability of connection in control: 21 out of 30 tested pairs, neuregulin treated: 25/30 pairs).

Taken together, we conclude that neurgulin is sufficient to accelerate the maturation of PV response properties via a mechanism independent of *de novo* L2/3 excitatory synapse formation onto PV neurons.

Experiment 2

Down-regulation of neurgegulin signaling via blocking ErbB4 receptor activity using EctoErbB4 was assessed by injecting EctoErbB4 into the ventricles. Using in-vivo recording, we found that EctoErB4 resulted in a decrease in normalized response amplitude (control: 1± 0.06, EctoErbB4 treated: 0.86±0.04) without impacting broad tuning (control OSI: 0.09±0.15, EctoErbB4 treated: 0.09±0.18).

These results indicate that the action of neuregulin is in part mediated by ErbB4 tyrosine kinase receptor signaling, but that other mechanisms are also involved. It is likely that in addition to synaptic properties, neuregulin impacts intrinsic membrane properties of PV neurons. Such a mechanism appears to operate independent of ErbB4 receptor activity. Given recent reports in the literature in which it is described that input-otput current injection curves of PV neurons are strikingly modulated by neuregulin signaling in-vitro, direct impact of neuregulin on ion channels is the candidate mechanism. This view is consistent with the results of Experiment 1 above.

Experiment 4
Mice in which the ErbB4 tyrosine kinase receptor was knocked out were used to further define that neuregulin is acting at ErbB4 receptors expressed specifically in PV neurons. This is an important control, because ErbB4 receptors are also expressed in another inhibitory cell type, the somatostatin-expressing neurons. Given our assays are performed in circuits composed of multiple cell types, we need to rule out the possibility that the effects we observed our not due to non-PV neurons.

We found that the effects of neuregulin treatment described in Experiment 1 results (Figure1a) were lost in the ErbB4 KO in which ErbB4 receptor was knocked out specifically in PV neurons. OSI index remained high, 0.19±0.17. Thus, ErbB4 receptor tyrosine kinase receptor expression in PV neurons is essential for neuregulin to mediate its effects on the maturation of PV neuron response properties.

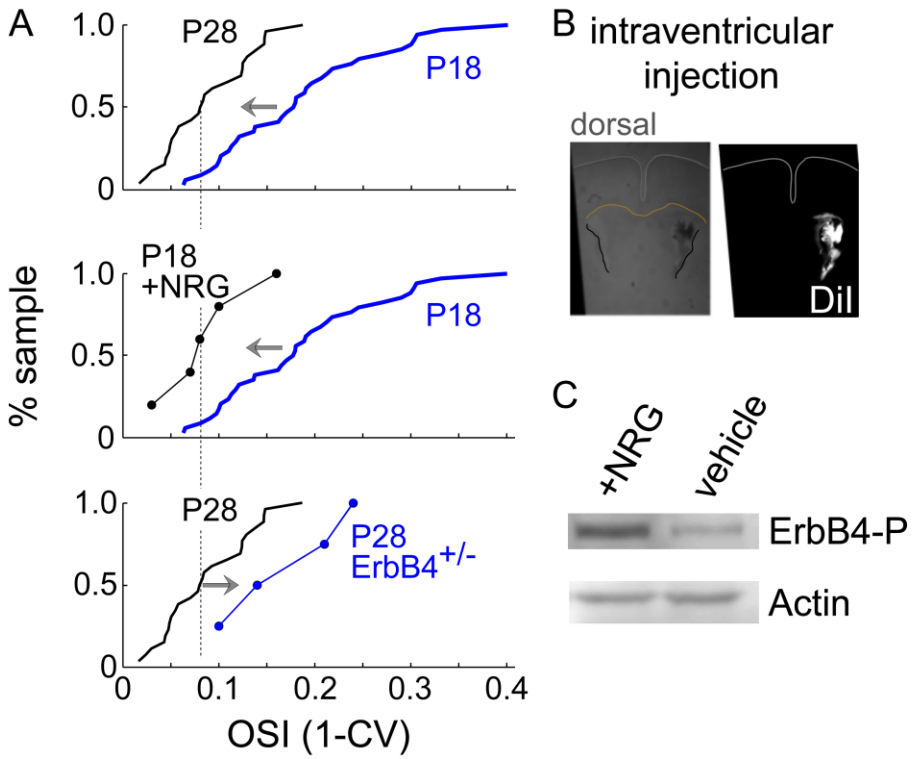

***Figure 1*. Impact of neuregulin-ErbB4 signaling on maturation of PV tuning.**
A, cumulative plots of OSI values of individual PV neurons recorded in young age group (P18) and mature (P28). Top, data re-plotted from Kuhlman et al. 2011. PV tuning broadens, represented as a leftward shift. Middle, PV neurons are more broadly tuned in animals injected with NRG (black, n=5 cells, 2 animals). Bottom, PV neurons fail to broaden their selectivity in PV-ErbB4+/- mice (blue, n=4 cells, 2 animals).
B, coronal brain section fixed immediately after a DiI/cresyl violet intraventricular injection as in Kuhlman et al. 2013 confirms location. Left, bright-field in which cresyl die is visible. Dorsal surface (gray line), corpus callosum (yellow line) and lateral borders of ventricle (black line) are indicated. Right, epifluorescence image.
C, Western blot demonstrates that ErbB4 is phosphorylated following an intraventricular injection of NRG- animal was sacrificed 3 hours after injection.