NSF EPSCoR Track-1
Strategic Plan

West Virginia Network for Functional Neuroscience and Transcriptomics (WV-NFNT)

35 WV STaR Division Science, Technology & Research

NSF Award #OIA-2242771
Principal Investigator: Juliana Serafin, Ph.D.
2023 - 2028

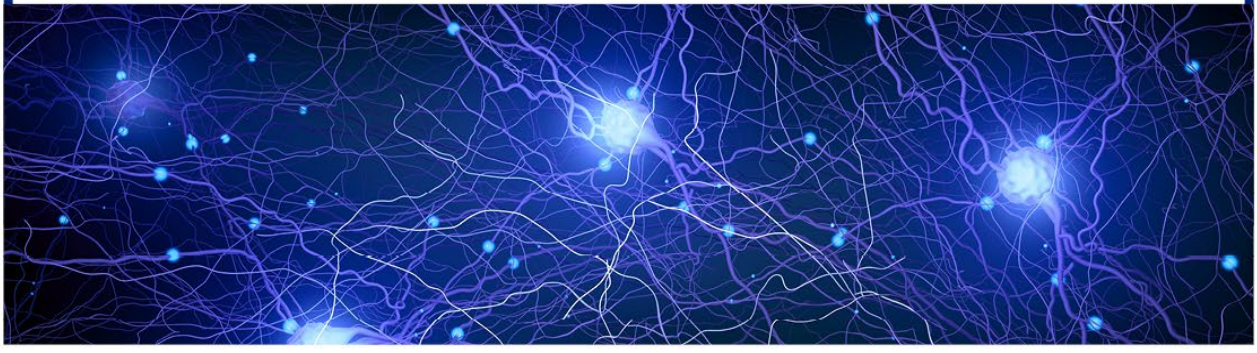

Table of Contents

The West Virginia Science and Research Council, established by the State Legislature in 2009, works to increase the capacity of the state and its colleges and universities to attract, implement, and use cutting-edge, competitive research funds and infrastructure. The Council also serves as the National Science Foundation's (NSF) Established Program to Stimulate Competitive Research (EPSCoR) jurisdictional steering committee for West Virginia with representatives of government, industry, business, and academia comprising its membership.

West Virginia was awarded a highly competitive, five-year $20 million grant from NSF EPSCoR in 2023 titled, "West Virginia Network for Functional Neuroscience and Transcriptomics (WV-NFNT)." WV-NFNT will establish a statewide collaboration of neuroscientists and bioinformaticists working to position West Virginia as a center for impactful neuroscience research.

The Council is responsible for developing and updating a strategic plan for this project. The new plan is presented in this document and provides a clear vision of how WV-NFNT will achieve its goals and overcome potential obstacles. The plan was approved and adopted by the Science and Research Council on September 27, 2023.

As stated in the Executive Summary, "The vision of the WV-NFNT is to transform neuroscience research in West Virginia by establishing connections and enabling access to the technologies and expertise needed for high resolution structure-function studies, and to build capacity and diversity through support for faculty, post docs, educators, and students, and to implement specific education and workforce development activities to engage underrepresented students especially first-generation college students in neuroscience research."

Through this project, West Virginia can reach even greater heights in research and innovation through our higher education community, benefiting our state's students, institutions, residents, and economy for years to come.

Sincerely,
Sarah Armstrong Tucker, Ph.D.

West Virginia Science & Research Council
NSF EPSCoR Jurisdictional Committee

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RII Track-1: West Virginia Network for Functional Neuroscience and Transcriptomics (WV-NFNT)

Award #: OIA 2242771

Vision: The vision of the WV-NFNT is to transform neuroscience research in West Virginia by establishing connections and enabling access to the technologies and expertise needed for high resolution structure-function studies, and to build capacity and diversity through support for faculty, post docs, educators, and students, and to implement specific education and workforce development activities to engage underrepresented students especially first-generation college students in neuroscience research.

Mission: Using different animal models, we will leverage infrastructure and human investments to answer fundamental questions about neural plasticity in two inter-related themes: 1) Circuit plasticity during development and adulthood, and 2) Synaptic structure and plasticity associated with altered function.

This Strategic Plan establishes the steps needed for successful implementation of the WV-NFNT project through specific goals, objectives, and actions. Outcomes, outputs, and a Risk Mitigation Plan are also necessary to achieve the vision and mission of the project.

WV-NFNT Project Management

The state Science & Research Council (SRC) is responsible for the state Science & Technology Plan and advises the WV EPSCoR office, the Science, Technology and Research Division (STaR) of the Higher Education Policy Commission (HEPC). The Executive Leadership Team (ELT), consisting of the PI, co-PIs, and Drs. Horstick, Sipe, and Antonsen, is responsible for project management and includes leads from Themes 1 and 2 and from the Education, Workforce Development and Diversity Team (EWDD), led by Co-PI Strait. The EWDD team will co-ordinate the named activities and interact closely with the ELT. Other groups that will play key roles in project management are the Expanded ELT for Resources (EELTR), the External Advisory Board (EAB), Industry Advisory Board (IAB) and the Evaluation Team (The Mark USA Inc.) Figure 1 shows the project management structure. A full description of each group can be found in the project proposal.

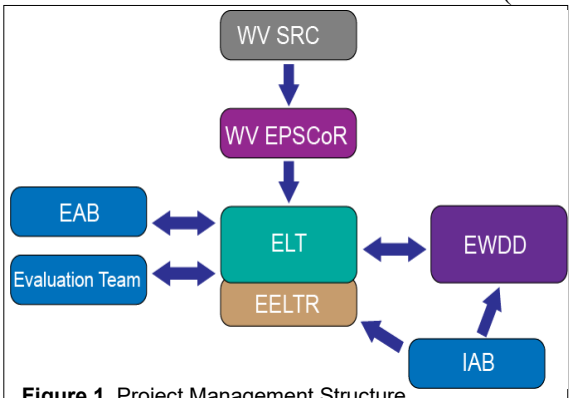

Figure 1. Project Management Structure.

Alignment with State S&T Plan

In 2021, a completely revised strategic plan, **Vision 2025: West Virginia Science and Technology Plan**, was created with input from more than 60 stakeholders from industry, higher education, and state government, and from the thorough analysis of data related to the research enterprise in the state. The plan was developed to build research infrastructure to attract federal research funding and research talent. The Science & Technology Plan has specific goals in five focus areas: **STEM Talent Pipeline, Research Enterprise, Innovation & Entrepreneurship, High-Tech Companies** and **Stakeholder Alignment.**

WV-NFNT will have a direct impact on the STEM Talent Pipeline and the Research Enterprise and will also influence the other focus areas. The research areas of neuroscience and transcriptomics are a strategic fit with the first two of the four science and technology platforms identified in the plan: **Life Sciences, Computer and Data Science, Advanced Manufacturing** and **Advanced Energy,** which were chosen based on the analysis of the research enterprise in the state and opportunities for growth**.**

Project Approach

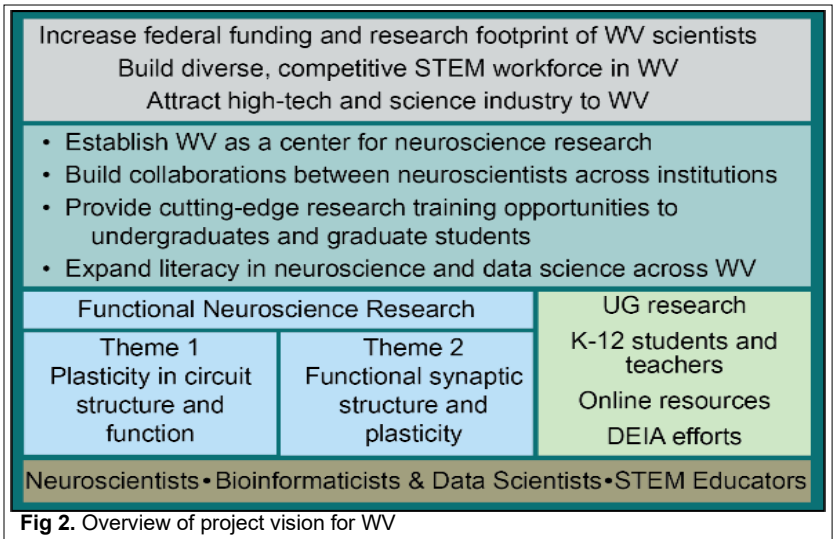

Fig 2. Overview of project vision for WV

WV-NFNT will build capacity and achieve the project vision by creating a network of neuroscience researchers working on high resolution structure-function studies in two-related themes: circuit and synaptic plasticity, and creating a related education, workforce development, and DEI effort, as shown in Figure 2. We will *build capacity* by making strategic faculty and staff hires, adding state-of the art microscopy and spatial transcriptomics infrastructure, and facilitating growth of the neuroscience community across the state. We will expand the capability and diversity of those working in the fields of neuroscience and bioinformatics through support for faculty, post docs, educators, and students, and by implementing specific education and workforce development activities to engage underrepresented students, especially rural, first-generation college students, in these research areas. Marshall University (MU), Shepherd University (SU), West Virginia State University (WVSU), and West Virginia University (WVU) are the four partner institutions at which capacity-building will be funded by the project.

The *overall scientific goal* of this proposal is to elucidate the structural underpinnings for functional connectivity and plasticity in diverse neural circuits. We will leverage infrastructure investments in high-resolution microscopy, transcriptomics, and expertise, to answer fundamental questions about neural plasticity in two inter-related Themes: ***Theme 1: Comparative circuit plasticity during development and adulthood***. We will address plasticity

under different developmental conditions, asking how circuits are remodeled through experience in different animal models (led by Dr. Horstick). ***Theme 2: Synaptic structure and plasticity associated with altered function.*** We will ask how ultrastructure and gene expression in neurons and glia contribute to synaptic functions in response to external stimuli or perturbations (led by Dr. Spitzer). Three cross-theme working groups (WGs) will be formed to support adoption of novel methodologies in individual research groups and foster collaboration: WG1: STED, led by Dr. Hruska; WG2: Transcriptomics, led by Dr. Horstick; WG3: Comparative Neuroscience, led by Dr. Sipe (**Table 4.3.1**). Figure 3 shows the research Aims for each theme and the animal models being studied, labeled as 1.x or 2.x in Figure 3.

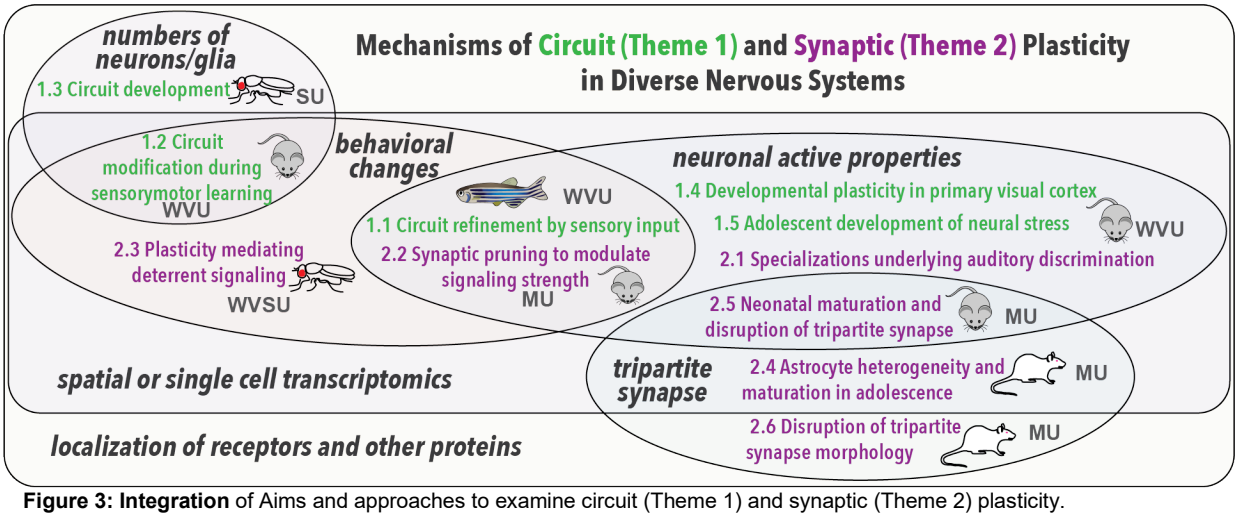

Figure 3: Integration of Aims and approaches to examine circuit (Theme 1) and synaptic (Theme 2) plasticity.

The Five Goals

The full proposal for WV-NFNT has been distilled into a set of five goals which provide the framework for the objectives, activities, outputs and outcomes, as well as the identification of risk mitigation strategies in this implementation plan:

Goal 1 Theme 1: Build jurisdictional capabilities in understanding circuit plasticity regarding functional connectivity between identified cell types as they emerge or are modified.

Goal 2 Theme 2: Build jurisdictional capabilities in understanding structure-function mechanisms underlying synaptic plasticity, that occur in response to external inputs or perturbations and contribute to generating and modulating the CNS.

Goal 3: Diversity: Use effective strategies to broaden participation in STEM among underrepresented groups (URG) and first-generation college students.

Goal 4: Education and Workforce Development: Improve preparation of students, teachers, and early career faculty to create a pipeline for the STEM workforce, using data science and neuroscience topics to generate interest in STEM.

Goal 5: Partnerships: Develop a cooperative, mutually beneficial relationship between researchers, stakeholder groups, and the community.

Research Timeline and Education/Workforce/Broader Impact Goals

Figure 4 gives ahigh level timeline for the work in the various Aims, the installation of new instrumentation, and faculty hiring.

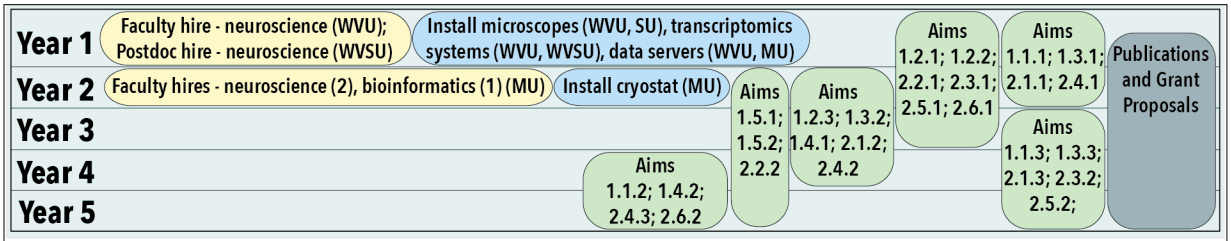

Figure 4. Timeline for infrastructure building and research activities.

Table 1 shows the number of participants that will benefit from the Education, Workforce Development, and Broadening Participation activities. This is inclusive of all project participants, including K-12 students and teachers, undergraduate and graduate students, and faculty at the 4 institutions. At the K-12 level this includes the development of new K-12 lessons in computer science and neuroscience, teacher trainings, and several summer camp series for middle and high school students (CodeWV, Teacher Training and Brain Camp, HSTA camps, and NYSF camps). In addition, professional STEM speakers will be engaged to encourage science enthusiasm and explore STEM career choices (GEAR UP). At the undergraduate/graduate student level this includes authentic research experiences, internships, course development, and diversity initiatives.

Table 1. Summary of education, workforce development, and broader participation impact

	K-12 Students	K-12 Teachers	Undergrad Students	Grad Students*	Faculty*
CodeWV	directly 1,000, indirectly up to 28,500, state-wide	950		1 (WVU)	4 (WVU)
Teacher Training & Brain Camp	56 (MU) 20 (SU) 100 (WVSU) 120 (WVU)	28 (MU) 20 (SU) 10 (WVSU) 50 (WVU)	10 (SU) 5 (WVSU) 35 (WVU)	3-4 (MU) 8 (WVU)	4 (MU) 3 (SU) 2 (WVSU) 20 (WVU)
HSTA Camps	400 (MU) 300 (WVSU) 160 (WVU)	4 (MU) 10 (WVSU) 16 (WVU)	16 (MU) 50 (WVSU) 16-20 (WVU)	8 (MU)	4 (MU) 4 (WVSU) 3 (WVU)
NYSF Camps	525	35	25 (state-wide)	4 (state-wide)	1 (HEPC)
GEAR UP	4,500	236			1 (HEPC)
Research & Research Mentoring			54 (MU) 30 (SU) 30 (WVSU) 65 (WVU)	3-4 (MU) 2 (WVSU) 7-9 (WVU)	5 (MU) 1 (SU) 2 (WVSU) 7 (WVU)
Interships			125 (all)		1 (SU)
Bioinformatics Bootcamp			50 (all)		2 (MU)
WV STEM Alliance URM			265 (state-wide, all 2&4 yr schools)		2 (MU)
CS Equity Curriculum			120 (MU) 125 (WVSU) 4,000 (WVU)		1 (MU) 1 (WVSU) 1 (WVU)

K-12 programs; Undergraduate & Graduate programs, * Graduate Students and Faculty per year, other data 5 year cumulative (but may in some cases include repeat partcipants year to year)

Strengths, Weaknesses, Opportunities and Threats (SWOT) Analysis and Areas of Special Emphasis in the Plan: Jurisdiction-Specific Programmatic Terms and Conditions (PTC) .

A SWOT (Strengths, Weaknesses, Opportunities, and Threats) analysis of the project for the five goals and Cross-cutting issues was used to identify internal weaknesses and external threats.

Team members then identified specific actions to mitigate these risks which are shown in the Risk Mitigation Plan table which is included after the Milestone Goals, Objectives and Actions.

The project was funded with three PTC's from the NSF. PTC 8.1 requires that WVU hire the Neuroscience faculty by the end of Year 2, and that Marshall hires two neuroscience faculty and one bioinformatics faculty by the end of Year 3. Goal 3 Objective 4.6.7 specifically includes these hires, but also includes objectives to attract more diverse candidates by advertising the postings at organizations targeting URM. Goal 4, Subgoal 4.4.3 embeds the hiring timelines as milestones. In the Risk Mitigation Plan, Risks 1 and 4 address having a potential lack of qualified candidates for the positions, and possible hiring freezes at WVU and Marshall.

Note that a recent development that may impact project finances at WVU is that indirect cost returns have been frozen or embargoed. It is unclear how long this situation will last, but it is addressed in Risk 5 in the Risk Mitigation Plan.

PTC 8.2 requires that goals, milestones and metrics for the implementation of MERSCOPE at WVU be included in the Strategic Plan and monitored throughout the project. Goal 1 Objective 1.6 addresses implementation milestones for the MERSCOPE, which are taken from a more detailed implementation plan developed by Dr. Horstick. Under Goal 1 and Goal 2, Aims 1.1, 1.4, 1.5, 1.6 and 2.1 also contains milestones for the use of the MERSCOPE in those projects. The Risk Mitigation Plan addresses MERSCOPE in Risks 2 and 8.

PTC 8.3 requires that the project provide an updated collaboration plan within the first 6 months of the project that provides milestones and metrics for collaboration in the first 2 years of the project regarding efforts to promote trainee involvement, and communication across the project and research efforts to promote cross-theme integration. Goal 5, Objective 3 specifically addresses cross-collaboration between themes and working groups for researchers, but the collaboration plan (to be submitted at a later date) will include more specific milestones and metrics. In the Risk Mitigation Plan, Risks 3 and 7 are related to this issue.

Another issue with the potential to negatively impact the project is not being able to recruit enough students (UG and Grad) to participate due to the declining enrollment and fiscal situations at the partner universities. Goals 1 and 2 include recruitment of students as researchers, and Goal 4 Subgoal 4.4.2 includes specific milestones for retaining undergraduate students. Risks 6, 18, and 22 address this issue in the Risk Mitigation Plan.

Goal 1: Address plasticity under different developmental conditions, asking how circuits are remodeled through experience in different animal models.

The ability of the nervous system to alter during development or in response to stimuli is fundamental to organisms' survival under changing conditions. Different conditions, developmental stages, and stimuli, result in functional changes to existing neurons that can alter physiological activity and organismal behavior. The molecular mechanisms and cellular interactions underlying this plasticity are incompletely understood and this gap in knowledge has been identified as a *grand challenge in neuroscience.* New methodologies to elucidate functional relationships underlying neural circuit activity are advancing our ability to examine how synapses, cells, and circuits are controlled at a level of detail not possible until recently. The WV-NFNT brings together researchers across WV who work on these questions and provides the infrastructure to address key gaps in knowledge about neural function.

We focus on diverse forms of experience-dependent plasticity that have broad implications for neural function and organism behavior. Our projects will examine how visual experience during key developmental windows reshapes neural function and molecular regulation using larval zebrafish (Aim 1.1). Cortical representations underlying texture dependent experience will be examined and how these experiences consolidate into memories using the mouse model (Aim 1.2). How dietary nutrient restriction impacts neural plasticity will be examined using *Drosophila* (fruit fly) as a model (Aim 1.3). In addition, mouse models will be used to examine how activity patterns s impacts visual experience dependent plasticity and circuit formation in cortical circuits (Aim 1.4) and how stress experiences remodel hypothalamic circuitry and function (Aim 1.5). Altogether Goal 1 addresses diverse forms of experience-dependent plasticity and capitalizes on unique strengths of various model systems.

Capacity building efforts for Goal 1 involve installation of a stimulated emission depletion (STED) microscope at West Virginia University (WVU) for super-resolution microscopy of fluorescently labeled proteins, cells, and cellular processes, providing the ability to resolve and quantify fine synaptic structure. Dr. Hruska (WVU, STED working group lead) has extensive experience with STED microscopy and will support the implementation of super-resolution microscopy hardware and guidelines for efficient and rigorous imaging experiments. Another major goal of the WV-NFNT is to build capacity for spatial transcriptomics – providing the ability to examine expression changes of hundreds of genes simultaneously with sub-cellular and precise in-situ spatial localization in biological samples. At WVU, we will incorporate a Vizgen MERSCOPE to provide spatial transcriptomics technology that is amendable to sub-cellular characterization and can accommodate experiments across the diverse array of species used in Theme/Goal 1. Dr. Horstick (WVU, Transcriptomics working group lead) has experience with characterizing RNA expression and will oversee the implementation of the MERSCOPE hardware and design of experimental protocols. MERSCOPE spatial transcritpomic profiling will be used to study plasticity in Goal 1 projects 1.1, 1.2, 1.4 and 1.5.

In Goal 1, we take an expansive approach that capitalizes on strengths of diverse model systems and brain regions to determine how different forms of environmental experience modulates neural circuits and imposes relevant changes in animal behavior.

Goal Theme 1: Build jurisdictional capabilities in understanding circuit plasticity regarding functional connectivity between identified cell types as they emerge or are modified.					
Objective Aim 1.1: Thalamic regulation of visual critical period plasticity and behavior in zebrafish					
Activity: *Aim 1.1.1: Determine how thalamus neurons regulate behavior.*					
Responsible: Horstick, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student(s)	X				
Train student(s) in lab techniques/fish care	X				
Generate constructs for tracing experiments		X			
Train in super-res imaging		X			
Perform tracing experiments (injections, imaging, registration)			X	X	
Identify downstream targets (ablation, others)					X
Circuit focused manuscript				X	
Submit grant			X		
Activity: *Aim 1.1.2: Determine how sensory experience regulates thalamic gene expression.*					
Responsible: Horstick, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit postdoc	X				
Train postdoc in lab techniques/fish care	X				
Train postdoc in MERSCOPE/MERFISH	X				
Validate section prep		X			
Design gene panel		X			
MERSCOPE/MERFISH experiments			X	X	X
Analysis				X	X
Transcriptomics manuscript					X
Submit grant including data				X	

Activity: *Aim 1.1.3: Assess whether molecular determinants of plasticity are conserved.*					
Responsible: Horstick, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Characterize transcript profile of plasticity driving neurons				X	X
Collect scRNAseq data from other models				X	X
Cross-reference expression patterns				X	X
Manuscript preparation					X
MERSCOPE/MERFISH using other species?					X

Objective Aim 1.2: The cortical circuits underlying texture discrimination in mice					
Activity: *Identify projection targets of texture engram cells*.					
Responsible: Agmon, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student	X				
Import TRAP2 mice from Jackson Laboratory	X				
Train mice in texture discrimination	X	X			
Retrogradely label layer 2/3 cells from S2 and M1		X	X		
Activity: *Identify changes in synaptic circuitry leading to engram formation*					
Responsible: Agmon, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student/postdoc		X			
Perform dual recordings from layer 2/3 neurons		X	X	X	
Activity: *Identify changes in gene expression patterns in engram cells*					
Responsible: Agmon, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Compare transcriptomic profile of TRAPed vs non-TRAPed neurons using MERFISH				X	X
Prepare and submit manuscript based on this aim				X	X

Objective Aim 1.3: Neural plasticity after developmental delay by dietary nutrient restriction					
Activity: *Aim 1.3.1: Quantify neuroblast proliferation and neuron/glia production after reactivation of neuroblasts from nutrient-induced quiescence.*					
Responsible: Sipe, SU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student(s)	X				
Train student(s) in *Drosophila* and confocal microscopy techniques	X				
Test and validate molecular markers	X	X			
Conduct starvation experiments and collect images of NSC productivity in larval/pupal brains		X	X		
Analyze images		X	X		
Activity: *Aim 1.3.2: Determine if the organization of nascent neurons into functional circuits in the adult brain is altered by nutrient restriction.*					
Responsible: Sipe, SU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student(s)		X			
Train student(s) in *Drosophila* and confocal imaging techniques		X			
Gather *vnd* fly lines, antibodies and validate	X	X			
Conduct starvation experiments and collect images of adult brains			X	X	
Analyze images for changes in axon number, axon bundling, arborization			X	X	
Activity: *Aim 1.3.3: Determine plasticity of synaptic structure during developmental interruption.*					
Responsible: Sipe, SU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student(s) (as necessary)			X	X	
Gather MARCM lines and validate generating single-cell clones			X	X	
Conduct starvation experiments in single-cell clone lines in combination with synaptic markers				X	X

Analyze images for changes in synapses				X	X
Prepare and submit manuscript based on this aim					X

Objective Aim 1.4: Development of the excitation/inhibition (E/I) ratio oscillation					
Activity: *Aim 1.4.1: Establish the developmental time course of the E/I oscillation*					
Responsible: Michelle Bridi, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit student(s)	X				
Train student(s) in whole-cell patch clamp	X	X			
Collect whole-cell recordings of inhibitory transmission across development		X	X		
Collect whole-cell recordings of excitatory transmission across development			X	X	
Manuscript preparation				X	
Submit grant				X	
Activity: *Aim 1.4.2: Determine the spatial and molecular identity of cells undergoing the E/I oscillation*					
Responsible: Michelle Bridi, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit postdoc		X			
Train postdoc in general lab techniques		X			
Validate section prep		X			
Design gene panel		X			
MERSCOPE/MERFISH experiments			X	X	
Analysis				X	X
Submit grant				X	
Manuscript preparation					X

Activity: *Aim 1.4.2: Determine the spatial and molecular identity of cells undergoing ODP*					
Responsible: Michelle Bridi, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Train postdoc in monocular lid suture			X		
MERSCOPE/MERFISH experiments			X	X	
Analysis				X	X
Manuscript preparation					X
Submit grant					X

Objective Aim 1.5: Development and vulnerability of hypothalamic stress circuitry					
Activity: Aim 1.5.1: Determine how PVN-CRH inputs and neuronal properties mature and are altered by stress experience.					
Responsible: Morgan Bridi, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit postdoc/student(s)/technician	X				
Train recruit(s) in patch-clamp techniques, animal care, stress & behavior	X				
Validate viral constructs and morphological reconstruction techniques	X	X			
Stress and patch-clamp experiments		X	X	X	
Analysis			X	X	X
Electrophysiology manuscript					X
Submit grant including data				X	

Activity: Aim 1.5.2: Investigate how programmed changes in gene expression and neuronal identity within PVN progress through development and are altered by experience.					
Responsible:					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit postdoc/student(s)/technician	X				
Train recruit in lab techniques, animal care, stress & behavior	X				
Train recruit in MERSCOPE/MERFISH	X				
Validate section prep		X			
Design gene panel		X			
MERSCOPE/MERFISH experiments			X	X	X
Analysis				X	X
Transcriptomics manuscript					X
Submit grant including data				X	

Objective 1.6: Capacity Building: Physical Infrastructure MERSCOPE					
Activity: Stage 1: Installation and setup at WVU					
Responsible: EH					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Vendor approval and supporting docs for ordering	X				
Establish space in genomics core	X				
Ordering and shipping	X				
Installation and training	X				
Create operations manual	X				
Activity: Stage 2: Sample prep and validation					
Responsible: Individual users					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Establish sectioning and maintaining intact tissue	X	X			
Show RNA labeling works	X	X			
Design Gene Panels		X	X	X	X

Activity: Stage 3: MERSCOPE/MERFISH experiments					
Responsible: Individual users					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Run MERSCOPE		X	X	X	X
Analysis		X	X	X	X

Objective 1.7 Capacity Building: Physical Infrastructure					
West Virginia University					
Activity: Install STED microscope at WVU					
Responsible: Horstick, Hruska, Nelson					
Sub-activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Order and install Leica Stellaris 8 STED system	X				
Integrate STED with research core	X	X	X	X	X

Goal 2: How do external inputs or perturbations alter synaptic structure to modify neuronal function?

Specialized synaptic interactions create the connections that allow neurons to communicate with each other and their targets. The ability to change synaptic strength is key to the formation and modification of neural circuits. For example, during circuit development and maturation, the process of synaptic pruning removes a subset of synapses, maintaining and strengthening others in response to neural activity.

Synaptic strength may be regulated via modulation of characteristic structures, including dendritic spine morphology on the postsynaptic cell where spine shape and size are associated with the strength of the connection or via the number, activity, or specific localization of receptors and other proteins in the spine. Synaptic plasticity also requires maturation and maintenance of specific interactions of presynaptic axons and postsynaptic dendrites with peripheral astrocyte processes. A *grand challenge in neuroscience* is to understand how neurons and synapses develop, connect, and change to create complex computational systems that allow organisms to successfully navigate their world. Goal 2 addresses this challenge by elucidating how structural changes occur in response to external inputs or perturbations and contribute to generating and modulating CNS function.

We will focus on neurons and changes in their subcellular synaptic connections to identify basic biological mechanisms of synaptic plasticity in response to exogenous input or perturbations. Sex-specific changes in synaptic structure and function in the auditory cortex in responses to pup calls (Aim 2.1) and in the prefrontal cortex in response to exogenous chemicals (Aim 2.2) will be examined in mice. Changes in gene expression patterns underlying behavioral responses to an environmental deterrent will be examined in *Drosophila* (Aim 2.3). Finally, we expand our approaches to include the astrocytic component of synaptic ultrastructure (the tripartite synapse) to determine the basic biological mechanisms underlying synaptic changes in response to exogenous chemicals (Aims 2.4-6) in mice or rats.

Capacity building efforts for Goal 2 involve installation of a stimulated emission depletion (STED) microscope at Marshall University for super-resolution microscopy of fluorescently labeled proteins, cells, and cellular processes, providing the ability to resolve and quantify fine synaptic structure. Dr. Hruska (Aim 2.1) is expert in this technique and four groups in Goal 2 will be incorporating this novel approach into their research programs (Aims 2.2, 2.4, 2.5, and 2.6). A data server and cryostat to support this instrument will also be installed and a technician will be hired. The services offered by the MU Genomics Core will be enhanced by adding the Visium (10x Genomics) platform; this approach will be used by two Goal 2 groups (Aims 2.4 and 2.5) to obtain a spatially resolved transcriptome analysis with broad coverage of mammalian genes followed by subsequent histology and imaging. WVSU will install a single-cell transcriptomics instrument (Nadia System) to analyze full transcriptomes in dissociated cells (Aim 2.3), this group has extensive experience in large-scale bioinformatics analysis. They will enter the field of neuroscience through this project, incorporating immunohistochemistry and confocal imaging to their research program. Finally, one group (Aim 2.1) will incorporate MERFISH spatial transcriptomics (See Goal 1).

In Goal 2, we will take a reductionist approach to examine how gene expression, morphology of synaptic components, and neuronal

activity underlie neural function and plasticity.

Goal 2 THEME 2: Synaptic structure and plasticity associated with altered function.					
Objective Aim 2.1: Determine the sex-specific molecular code of functional and structural neuronal connectivity in mouse auditory cortex (AC).					
Activity: Aim 2.1.1: Determine functional differences in the temporal AC of awake male and female mice.					
Responsible: Hruska and Anderson, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students	X	X			
Train students in functional microscopy	X	X	X		
Optimize stimulation parameters		X	X		
Data collection	X	X	X	X	
Manuscript prep/publication			X	X	X
Activity: Aim 2.1.2: Quantify changes in gene expression associated with functional differences.					
Responsible: Hruska and Anderson, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students for spatial transcriptomics	X	X			
Training using MERSCOPE set up	X	X			
Establish criteria for rigorous sample validation and analysis on MERSCOPE	X	X			
Gene panel design/sample collection		X	X		
Spatial transcriptomics experiments on functionally identified tissue		X	X	X	
Manuscript preparation/publications				X	X
Activity: Aim 2.1.3: Assess sex-dependent changes in specific proteins and their transcripts in dendritic spines identified to correlate with auditory inputs.					
Responsible: Hruska and Anderson, WVU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students for spatial transcriptomics	X	X			
Training on STED microscope	X	X			

Sample collection		X	X		
STED experiments on functionally identified tissue		X	X	X	
Manuscript preparation/publications				X	X

Objective Aim 2.2: Evaluating the impact of early exposure to nicotine on long-term addiction-related behaviors					
Activity: Aim 2.2.1 Test the hypothesis that early exposure during adolescence will decrease synaptic pruning in PFC neurons that project to the VTA.					
Responsible: Henderson, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Optimization of RNAscope (spatial transcriptomics)	X				
Recruitment/training of students in RNAscope	X				
Brain slice electrophysiology of mPFC	X	X	X		
Nicotine drug self-administration	X	X	X		
Publication		X	X		
Activity: Aim 2.2.2. Determine if adult mice exposed during adolescence will exhibit an increased risk for addiction- related behaviors as measured by drug self-administration.					
Responsible: Henderson, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Nicotine Drug Self-administration		X	X	X	X
mPFC dendritic spine morphology			X	X	
Publication			X		X
Objective **Aim 2.3: Resolving capsaicin-induced neuronal effects through comparative spatial transcriptome**					
Activity: Aim 2.3.1: Examine if LOF mutants on capsaicin diet have altered spatial transcriptomes in brain or modified motor behavior.					
Responsible: Reddy, WVSU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Spatial sequencing of brain sections of wild types on capsaicin diet	X				
Standardizing bioinformatic pipelines for brain spatial sequencing	X	X			

Creation of LOF mutants for 14 genes using TRiP-CRISPR knockout technology and spatial transcriptome changes for the mutants that manifest modified motor behavior	X	X	X		
Recruitment of postdoc	X				
Recruitment of students	X	X	X	X	
Creation of independent research projects for the students and training		X			
Mating LOF mutants for creating double and triple mutants			X		
Optimize IHC with the confocal microscope at MU.		X	X	X	
Peer reviewed publications		X	X		
Capacity Building Proposal for Confocal microscope	X	X	X		
Activity: Aim 2.3.2: Determine if expression patterns change in LOF mutants on capsaicin diet.					
Responsible: Reddy, WVSU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Examine epistasis of double and triple mutants in motor behavior under capsaicin diets			X	X	
Studies on protein expression patterns change in LOF mutants			X	X	X
Student recruitment and training		X	X	X	X
Peer Reviewed publications		X	X	X	X
Proposal writing		X	X	X	X

Objective Aim 2.4: The effects of adolescent binge drinking on tripartite synapse integrity					
Activity: Aim 2.4.1: Quantify changes in astrocyte heterogeneity and maturation across subregions of the OFC throughout adolescent development.					
Responsible: ML Risher, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students	X	X			
Train students in animal handling, AAV, IHC	X	X			
Optimize Visium workflow	X				
Sample Collection	X	X	X		

Imaging/Analysis	X	X	X		
Submit peer reviewed manuscript			X		
Activity: Aim 2.4.2: Assess the relationship between dendritic spine characteristics and loss of astrocyte-synaptic proximity within sub-regions of the OFC following adolescent binge ethanol exposure.					
Responsible: ML Risher, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students		X	X	X	
Train students in animal handling, AAV, IHC		X	X	X	
Sample Collection		X	X	X	
Imaging/Analysis		X	X	X	
Submit peer reviewed manuscript				X	
Activity: Aim 2.4.3: Assess changes in the transcriptome that are spatially relevant for astrocyte-synaptic stabilization.					
Responsible: ML Risher, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students			X	X	
Train students in animal handling, AAV, IHC			X	X	
Sample Collection			X	X	
Imaging/Analysis			X	X	X
Submit peer reviewed manuscript			X	X	X

Objective **Aim 2.5: Characterize the effects of early life opioid exposure on neuronal and astrocytic structural synaptic development (C. Risher, MU).**					
Activity: Aim 2.5.1: Quantify changes in tripartite synapse ultrastructure and gene expression patterns**.**					
Responsible: C Risher, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students	X	X	X	X	X
Train students in animal handling, AAV, IHC	X				
Optimize Visium workflow	X				
Sample Collection	X	X	X		

Imaging/Analysis	X	X	X		
Submit peer-reviewed manuscript			X		
Activity: Aim 2.5.2: Assess changes in synaptic function associated with structural alterations.					
Responsible: C Risher, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Electrophysiology (with Henderson lab)			X	X	
Data analysis			X	X	X
Submit proposal				X	
Submit peer-reviewed manuscript					X

Objective **Aim 2.6: Disruption of tripartite synapse by low-level silver nanoparticles (AgNPs) in the striatum**					
Activity: Aim 2.6.1: Is protein expression in pre-and post-synaptic structures in the striatum changed by AgNP exposure?					
Responsible: Spitzer, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruit students	X	X	X	X	X
Train students in IHC and confocal microscopy	X	X	X	X	
Optimize IHC and imaging protocols	X				
Data collection and analysis	X	X	X		
Submit peer reviewed manuscript			X		
Activity: Aim 2.6.2: Does AgNP treatment alter ultrastructural morphology of striatal tripartite synapse components?					
Responsible: Spitzer, MU					
Sub-Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Develop collaboration with ML Risher Lab		X	X		
Train PI and students in AVV vector techniques		X	X	X	
Optimize labeling and imaging protocols		X	X		
Data collection and analysis			X	X	X

Submit proposal				X	
Submit peer reviewed manuscript					X

Objective 2.7 Capacity Building: Physical Infrastructure					
Marshall University					
Activity: Install STED microscope, cryostat and data server at MU					
Responsible: Antonsen / Norton / Malik					
Sub-activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Order and install Leica Stellaris 8 STED system	X				
Order and install new Leica cryostat		X			
Hire a full-time technician to oversee the STED system, train students, and contribute to sample processing	X				
Form steering group to develop and manage STED training, data management, fee structure, and related systems	X	X	X	X	X
Data server installed	X				

Goal 3: Diversity: Use effective strategies to broaden participation in STEM among underrepresented groups (URG) and first-generation college students

At the university level, the focus will be on retention of first-generation college students in STEM majors, targeted recruitment of URG for undergraduate research, the development of the WV STEM Alliance for URM thereby expanding WV's limited LSAMP reach, and by increasing diversity with proposed new faculty hires. Broadening participation will be supported at the K-12 level through the equity focus of CodeWV and summer camp programs.

Goal 3: Diversity – Use effective strategies to broaden participation in STEM among underrepresented groups (URG) and first-generation college students. (Note that in the section below, the number refers to the proposal numbering)					
Objective: 4.6.1 – Establish WV STEM Alliance for URM					
Responsible: D. Cartwright, Quinones					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Host WV STEM Alliance Symposia			X	X	X
Recruit new students to join LSAMP network	20	25	30	35	40
Objective: 4.6.2 – Use existing HSTA infrastructure to excite HS students					
Responsible: D. Cartwright					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Incorporate Neuroscience/Data Science modules in HSTA programs to train teachers		10	10	10	10
Utilize Neuroscience/Data Science modules with students during HSTA		60	60	60	60
Objective: 4.6.3 – Broaden Participation in undergraduate research opportunities					
Responsible: Horstick, Spitzer, Reddy, Sipe					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Percentage of undergraduate researchers who are first-generation		50%	50%	50%	50%
Percentage of undergraduate researchers who identify as female		50%	50%	50%	50%

Percentage of undergraduate researchers who are African American/Black		5%	5%	5%	5%
Percentage of undergraduate researchers who are Latinx		4%	4%	4%	4%
Objective: 4.6.4 – Broadening participation in graduate research opportunities					
Responsible: Nelson					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Recruitment of faculty advisor	X	X	X	X	X
Recruitment of NURO students		X	X	X	X
Broadening of NURO program to other WV institutions		X	X	X	X
Assessment of success of grad school placements			X	X	X
Objective: 4.6.5 - Diversify the computer science K-12 pipeline through CODEWV					
Responsible: Stewart, Licwov-Channell					
Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Increase percentage of students who have access to Equity focused CS courses through CODE.Org curriculum implementation (current baseline 44% of students have access to such a course)		50% access	60% access	70% access	80% access
Objective: 4.6.6 – Diversify the computer science college pipeline through inclusion of P4E (partnership for equity) curriculum into introductory computer science courses					
Responsible: Malik, Payne					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Students using P4E at WVU		800	800	800	800
Students using P4E at MU		24	24	24	24
Students using P4E at WVSU		50	50	50	50
Objective: 4.6.7 – Increase diversity of new faculty hires					
Responsible: Murphy, Antonsen					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Post job ads and send to specific organizations targeting URG	X				
Hire one tenure track faculty at WVU, 3 at MU, and one postdoc at WVSU		X			
Hire 3 postdocs at WVU (unspecified dates)		X	X	X	X

Follow mentoring plan, with attention to factors critical to URM success		X	X	X	X
Encourage attendance at the Southern Regional Education Board Institute on Teaching and Mentoring for new hires, faculty mentors, and others as appropriate			X		

Goal 4: Education and Workforce Development: Improve preparation of students, teachers, and early career faculty to create a pipeline for the STEM workforce, using data science and neurosciences to generate interest in STEM

WV-NFNT, while focusing on neuroscience, will have a major impact on the STEM pipeline: 1) expanding K-12 opportunities for students and teachers; 2) partnering with and expanding existing programs (e.g., INCLUDES First2, Health Sciences & Technology Academy [HSTA], CodeWV, S-STEMs, Louis Stokes Alliance for Minority Participation in WV and Kentucky [KY-WV LSAMP]) to prepare and retain college students; and 3) partnering with companies and labs to increase internships. A significant portion of the research component of this project will be conducted by an undergraduate work force. Formalized training and mentorship of undergraduates in neuroscience and data science research, coupled with access to internships, will contribute to the goal of placing West Virginians in competitive post-graduate programs or employment in STEM industries.

Goal 4: Improve preparation of students, teachers, and early career faculty to create a pipeline for the STEM workforce, using data science and neuroscience topics to generate interest in STEM.					
Subgoal 4.4.1 Expand the data science and neuroscience reach to K-12 students and educators					
Objective: 4.4.1.1-High quality teacher professional development through CODE WV					
Responsible: Stewart/Licwov-Channell					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Facilitators and research experts work together to use transcriptomics data to develop use guidelines to allow HS teachers and undergraduates to explore the data.	X				
New lessons developed related to the research will be made available to computer science teachers for use in their classroom.		5	5	5	5
NEW HS data science course fully developed					X
Engage additional 750 elementary teachers		100	200	225	225
Engage additional 100 middle school teachers		12	20	30	38
Engage additional 100 high school teachers		12	20	30	38

K-12 classes will have a transcriptomics research project embedded in their curriculum		5	10	15	20
Objective: 4.4.1.2 – Immerse in-service educators in training and development of neuroscience/data science summer camps and Project Based Instruction					
Responsible: Carver, T. Cartwright, Payne, Wang					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Develop relationships with research scientists for development of k12 workshop/camp activities	X	X	X	X	X
Engage teachers in PBI/Neuroscience/Data science camp at WVU		3-4	3-4	3-4	3-4
Engage teachers in PBI/Neuroscience/Data science camp at MU		4	6	8	8
Engage teachers in PBI/Neuroscience/Data science camp at SU		8	8	8	
Engage teachers in PBI/Neuroscience/Data science camp at WVSU		2	2	3	3
All lessons developed will be made publicly available to teachers (Teachers paired for lesson development)		9	10	12	8
All Virtual professional development sessions will be available to all WV educators through WVDE		X	X	X	X
Objective: 4.4.1.3 – Engage HS teachers and students in neuroscience/data science at Brain Camp					
Responsible: Carver, Licwov-Channel, T. Cartwright, Payne, Wang					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Engage students in Brain Camp at WVU	25	25	25	25	25
Engage students in Brain Camp at MU		8	12	16	16
Engage students in Brain Camp at SU		8	8	8	
Engage students in Brain Camp at WVSU		25	25	25	25
Objective: 4.4.1.4 – Science Camps targeting URM and deeply rural students in partnership with National Youth Science Academy					
Responsible: Strait					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Neuroscience camp in Huntington, WV		12	15	20	24
Neuroscience camp in Charleston, WV		12	15	20	24

Neuroscience camp in Fayetteville, WV		8	12	15	20
Neuroscience camp in Davis, WV		8	12	15	20
New camp location			12	15	20
Objective: 4.4.1.5 – STEM Speakers Series collaboration with WV GEAR UP					
Responsible: Strait					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Annual GEAR UP Speaker event student attendance	1,000	1,000	750	500	90
Annual GEAR UP Speaker teacher attendance	60	60	50	45	20
Pre-speaker activities	1,000	1,000	750	500	90

Subgoal 4.4.2 – Increase retention of undergraduate students through research and mentoring, particularly in the first two years of college.					
Objective: 4.4.2.1 – Incorporate a formal mentoring structure and support early-career undergraduate research in neuroscience and data science					
Responsible: T. Cartwright, Payne, Quinones, Sipes, Stewart					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Develop and enhance the capacity of the Research Rookies program by implementing work-study opportunities, following the successful model of WVU RAP (WVU)		X	X	X	X
Develop and disseminate structured mentoring modules for undergraduate students (WVSU, MU)		X	X	X	X
Create Independent development plans to track students and measure research skills, scientific knowledge, communication, and independence/professionalism (WVSU, MU)		X	X	X	X
Support students in a research mentoring program (Research Apprenticeship Program) (MU)	4	6	8	8	8
Support students in a Research Rookies mentoring program (WVSU)	2	2	2	2	2
SU supports undergraduate students (send to MU or WVU for research experiences)	2	2	2	2	2

WVU Support neuroscience/data science faculty involved in the proposed work in engaging undergraduate researchers in that work, building upon the infrastructure of the RAP program		10	10	10	10
Objective: 4.4.2.2 – Development of internship opportunities with local industries.					
Responsible: Wang, Strait					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Create/Develop workplace Internship opportunities for students		10	15	20	25
Advertise Internships to relevant students		X	X	X	X
Create Relationships with Local Industries for Internships	9	9	9	9	9
Develop internship/job/research lab portal	X	X	X		
Activity: 4.4.2.3 – Bioinformatics Bootcamp					
Responsible: Denvir, Strait					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Students attend bioinformatics boot camp		5	5	5	5
Reach out to institutions for recruitment		16	16	16	16

Subgoal 4.4.3 Capacity Building: Human Infrastructure					
Objective: MU Three faculty hires – 2 Neuroscience, 1 Bioinformatics					
Responsible: Antonsen					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Post job ad and conduct interviews	X				
Hire three Assistant Professor level tenure-track faculty members		X			
Implement mentoring plan		X	X	X	X
Objective: WVU One faculty hire – 1 Neuroscience					
Responsible: Nelson					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Post job ad and conduct interviews	X				
Hire Assistant Professor level tenure-track faculty		X			

Implement mentoring plan		X	X	X	X

Objective: Develop formal early career mentoring plan for new faculty hires and postdocs					
Responsible: Nelson, Antonsen, Reddy					
Activities	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Refine formal WVU mentoring system as applicable to WVSU and MU College of Science, and coordinate with MU School of Medicine	X				
Identify faculty mentors for new MU hires and develop decision tree.		X			
Document progress towards tenure for MU faculty, identify opportunities, resources, and challenges/solutions as needed or applicable.		X	X	X	X
Coordinate with WVSU to ensure new postdoc hire has access to resources and opportunities through WVU and MU	X				
Identify additional early career faculty appropriate to apply for seed funding at WVU and MU, ensuring that they have access to mentoring resources		X (WVU only)	X	X	X (MU only)
Assess WVSU postdoc for promotion to tenure track faculty			X (end of Year 2)		

Goal 5: Partnerships: Develop a cooperative, mutually beneficial relationship between researchers, stakeholder groups and the community

WV-NFNT will build partnerships in several ways to expand the impact and sustainability of the project. An Industry Advisory Board (IAB) will provide an opportunity for industry representatives to engage with researchers to foster collaboration and technology transfer. It will also provide an important opportunity for trainees to learn about careers in neuroscience/data science and to develop their professional skills by interactions with IAB members. Collaborations between faculty and students at the four institutions (MU, SU, WVSU, and WVU) will be strengthened to the benefit of neuroscience research and education in the state. Very specific activities are included here and in the collaboration plan to promote the success of the project by making sure that researchers are not siloed in individual labs or themes. Another key partnership to the project is that with the community at large. The engagement and participation of students and citizens is key for successful implementation of the goals.

Other key partnerships not mentioned here are included in Goals 3 and 4.

GOAL 5: Partnerships - Develop a cooperative, mutually beneficial relationship between researchers, stakeholder groups, and the community.					
Objective 1: Create Industry Advisory Board and engage them with the project themes for the benefit of students and researchers					
Responsible: Serafin/Horstick					
Activities:	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Identify companies for IAB membership, and add new companies as appropriate	X	X	X	X	X
Hold Technology Transfer Workshop for Researchers	X	X	X	X	X
Develop academic-industry partnerships		X	X	X	X
Develop internships for UG / Grad students and opportunities for students and industry to interact (Internship Portal in EWDD)		X	X	X	X
Objective 2: Build multiple partnerships and collaborations based upon the contributions and connections between the partner institutions: WVU, MU, SU, and WVSU.					
Responsible: Antonsen / Nelson / Fultz /Sipe					
Activity	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**

Faculty in the project visit and collaborate with other campuses in the project		X	X	X	X
UG and Graduate students in the project visit other campuses in the project		X	X	X	X
20% of UG, 50% of grad students, 100% of postdocs present or are authors on a poster at All Hands Meetings			X	X	X
Build connections between URM and First Gen students at campuses		X	X	X	X
Send advertisement for seed funding opportunity to appropriate units and institutions (WVU, MU, WVSU, SU)	X	X	X	X	
Project seed funding is awarded to researchers collaborating from multiple institutions and/or between themes, EWDD. >50% of approved projects are cross-institutional or involve multiple theme/WGs by Y3 (WVU) and Y4 (MU).		X WVU	X MU X WVU	X MU X WVU	X MU
Objective 3: Create cross-collaboration between Themes and WGs					
Responsible: ELT / PI					
Activities:	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
Structure meetings to promote collaboration and create peer cross-institutional networks. Student and postdoc participation in meetings increase and 2 or more co-authored proposals / publications by Y3 from theme/WG collaborations.	X	X	X		
Create concept maps for overall project, themes and WGs to help participants visualize connections and interdependencies during first 6 months of project / and update collaboration plan yearly	X	X	X	X	X
Create and use Shared Resources Drive including "Ask me about" Directory, "Ideas Whiteboard" and "Milestones". All meeting notes and agendas are on the One Drive.	X	X	X	X	X
All researchers contribute to quarterly reports which meet quality and deadline criteria.	X	X	X	X	X
Training is offered to expand research capabilities. Relationships to Genomics Cores are strengthened.	X	X	X	X	X
Objective 4: The community is aware of and participates in project activities.					

Responsible: HEPC					
Activities:	**Y1**	**Y2**	**Y3**	**Y4**	**Y5**
The Neuron, video series, website, speaker series, quarterly email newsletter, social media campaigns are used to engage the community	X	X	X	X	X
Participation in K-12 education activities, camps and other activities meets attendance targets	X	X	X	X	X
Partner institutions (WVU, MU, WVSU and SU) publicize the project.	X	X	X	X	X
The project contributes to national-facing web-publications at least yearly	X	X	X	X	X

WV-NFNT Project Management Milestones
(Note: Q1 of Year 1 starts June 1, 2023)

Task	Y1	Y2	Y3	Y4	Y5
Theme 1: MERFISH Acquisition & Validation	Q2				
Recruit MERFISH postdoc	Q4				
Recruit undergraduates for summer NURO	Q4				
Recruit 1st year grad students for research labs		Q4			
Train lab personnel in MERFISH			Q1		
Theme 2: Develop Theme 1 quarterly report template	Q1				
Training and consultation from Dr. Morganti (UK) for Dr. ML Risher on 10x Genomics Visium platform	Q1& Q2				
Develop protocols, sample workflow, and have imaging training for Dr. C Risher and Dr. ML Risher from Dr. Hruska	Q2				
Training from Dr. Hu for other team members	Q2				
Training from Dr. ML Risher for Dr. Spitzer on AAV vectors		Q1			
EWDD: Identify biotech and neuroscience company contacts for internship portal	Q1				
Identify theme and topics for one week camp	Q1				
Secure neuroscience speakers for Marshall Research – Mentoring Course	Q2				
Develop content for WVSU Brain Camp	Q2				
Get info from researchers on how K-12 teachers can access transcriptomics data for WVU	Q2				
Get list of skills / content for K-12 students that would be useful from researchers for WVU	Q2				
Develop details of sending Shepherd UG students to WVU, including the labs they will work in	Q3				
Neuroscience demo for Science Olympiad at Marshall	Q3				
Neuroscience faculty and grad students teach and present at Brain Camp		Q1			
Administration (HEPC, ELT): Order MU STED (Antonsen)	Q1				
Hire technician for STED at MU	Q3				
Install MU STED	Q3				
Teams to deliver info for Annual Report to HEPC	Q3				
ELT to plan All Hands Meeting and request presentations	Q4				
Attend EPSCoR PI Meeting in May	Q4				
MERFISH implementation plan included in Strategic Plan (PTC 8.2)	Q1				
Submit updated collaboration plan including cross-theme integrations for trainees to NSF (PTC 8.3)	Q2				

Prepare for Year 2 and Year 4 Reverse Site Visit from NSF		Q2		Q2	
Marshall to hire 3 faculty (PTC 8.1)		Q4			
WVU to hire 1 faculty (PTC 8.1)		Q4			
Antonsen to recruit graduate students for Marshall	Q4				
Request NCE from NSF for Y5 summer programs					Q3

Risk Mitigation Plan

A SWOT analysis of the project which included the five goals and Cross-cutting issues was used to identify internal weaknesses and external threats. Team members then identified specific actions to mitigate these risks which are shown in the Risk Mitigation Plan table. In identifying responsibility, the initials of the institution are used in cases where parallel actions are required at more than one institution. Otherwise, the institution is identified in the action or is not relevant.

RISK MITIGATION PLAN					
Number	Risk	Risk Likelihood	Risk Impact	Major Actions or Mitigation Activity for High Likelihood Risks	Responsibility
		High	High		
		Medium	Medium		
		Low	Low		
Risk Category: Overall Project Cross-cutting Issues					
1	Faculty hiring packages do not attract qualified candidates.			Leverage research offices, colleges, departments, and academic affairs as needed to create best possible offer.	Antonsen (MU), Nelson (WVU)
2	MERFISH and other transcriptomics techniques are difficult to implement and use.			Specific implementation plan for MERFISH is shared and used by researchers. Transcriptomics new hire in MU School of Medicine will focus on the techniques and work with external collaborators to help researchers use techniques.	Horstick (WVU) / Spitzer (MU) / New MU Transcriptomics hire
3	Lack of communication leads to siloing of work into themes and/or individual research groups.			Meeting and reporting practices allow researchers to leverage across themes / working groups. Collaboration plan, communication among students and seed funding designed to prevent siloing.	Team/working group leads (Spitzer/Horstick/Hruska/Sipe)
4	Hiring freezes at universities slow down faculty hires required by 8.1 PTC (1 WVU, 3 MU).			Designate person at each university to work with administration; consider if and when to involve NSF.	Serafin / Murphy (WVU)/ Antonsen (MU)

5	WVU freezes or limits the use of indirect cost funds and/or startup funds that were expected to contribute to the project.			Make sure that WVU Research Office of Sponsored Programs continues to champion for the release of funding.	Murphy (WVU) / Nelson (WVU)
6	Challenges recruiting talented and motivated students & postdocs.			Form a strong internal pipeline to recruit and move students through undergrad and grad opportunities, along with strong external links and advertising like recruiting fairs at SFN.	Antonsen (MU)/Nelson (WVU)
Risk Category: Goal 1 Theme 1					
7	WG2 MERFISH implementation not established.			Clarify resources and optimize resource-sharing; leverage company training, and local expertise building within project. Consider employing Vizium system at MU.	Bridi/Horstick
8	Siloing within Theme 1			Monitor level of collaboration, encourage communication, and take corrective action.	Horstick
Risk Category: Goal 2 Theme 2					
9	Resolution of STED is not adequate.			Include expansion microscopy if necessary. Active WG 1 to ensure best practices in sample preparation are adopted.	Hruska, C Risher
10	Aims 2.3 2.5 2.6: Novelty of Visium spatial transcriptomics, confocal, STED and AAV approaches are barriers to productivity. No confocal available at WVSU.			Training with WVU Genomics core. Expertise of external consultant, Dr. Morganti for transcriptomics. WVSU writing grant for confocal. Collaborations will address novelty issues.	Experts in each technique
11	Aim 2.1.1: It is possible that the GCaMP 6-7 generations might not be fast enough to obtain single synapse output of auditory behaviors.			Use a faster version (e.g., GCaMP8-fast) or voltage sensitive dyes (e.g. Voltron) that have been successfully optimized in vivo to record output of cell bodies and synapses. Optimize this if this becomes challenge.	Hruska/ Anderson

12	Aim 2.1.2 and 2.1.3: Combining STED and expansion microscopy of single proteins/mRNAs might result in low signal to noise ratio due to the methodology of labeling molecules in crosslinked, expanded tissue			Optimize this if this becomes a challenge. Alternatively, we will used well defined targeted RNA quantification approaches.	Hruska/ Anderson
13	Aim 2.2: Immunofluorescence will provide insufficient signal to allow morphological measurements of dendritic spines via STED.			PFC cells will be filled directly with lucifer yellow.	Henderson
14	Spitzer lab may be closed by Science Building Renovation			MU COS is working to secure flex space to house displaced labs during the renovation.	Antonsen
15	Potential renovations in VA may close L. Risher space.			Move animals to MU ARF if necessary.	ML Risher
Risk Category: Diversity					
16	Few URG included in research teams.			Include diversity considerations on hiring new research staff.	Antonson (MU), Nelson (WVU)
17	Recruiting timeline does not allow for developing team diversity.			Work with CODEWV and WVDE to create timelines for recruiting.	Licwov-Channell/ Stewart
18	Few URG included as mentors.			Use mentors from affinity groups in addition to research mentors (INCLUDES, LSAMP, Women in Computing, IEEE).	D. Cartwright, Stewart, Bright
19	DEI offices and other supportive units at universities at risk by politics/culture war.			If DEI offices are disbanded look to other university resources.	Strait
20	Some counties resist training teachers because of lack of personnel and opportunity to fit coding into schedule (increase CODEWV access).			Work with counties to help them understand that coding can be counted as a math or science course for WV students.	Licwov-Channell/ Stewart

Risk Category: Education & Workforce Development					
21	UG Research Each institution has variation in expertise and resources; limited areas of expertise and resources for smaller institutions.			Find out if researchers at other institutions need smaller pilot studies done that we could help with.	Preterotto/ Bright
22	UG Research: Research project depends on undergraduates who will have relatively little research experience and potentially varying levels of commitment.			Implement training for research at Shepherd University.	Sipe
23	There are not many companies entirely focusing on neuroscience and many large companies with neuroscience components are located in CA/FL which are far for WV students to go for internship opportunities.			Maybe consider providing some financial assistance (such as travel stipend/award) to encourage students take internship opportunities far from WV.	Wang
24	Minimal networking between EOD team and researchers.			Devise and implement activities designed to build relationships. Develop regular communication between EWDD group and research group.	Strait
25	CodeWV: National CODE.org network reports low participation in PD for k12 teachers.			Work with WVDE to engage with local school leadership to encourage broader participation.	Licwov-Channell
26	Brain Camp Teacher PD on PBI was to be funded through indirect costs returned to the Neuroscience Center. Indirect funds have been frozen by the university due to overall budget constraints.			Lobby for restoration of indirect costs if they are still frozen in Year 2, and look for other sources of funding, perhaps through state grants.	Carver, Licwov-Channell, Nelson (WVU), Serafin (HEPC)
27	No content experts on EOD team.			Build relationships with content experts.	Strait
28	CodeWV will need to coordinate efforts to develop new materials among several teams.			Develop a timeline early.	Licwov-Channell
29	Code WV: Some WVDE counties are harder to reach with new materials.			Work with WVDE to engage with local school leadership to encourage broader participation.	Licwov-Channell
30	Saturation of the market with professional development opportunities for high school teachers.			Create recruiting materials early, consider budget options for distance recruiting.	T. Cartwright, Carver, Licwov-Channell, Wang

31	Potential high school teacher burnout post pandemic.			Blended format for teacher workshop with break between remote and face to face activities.	T. Cartwright, Carver, Licwov-Channell, Wang
32	High school teachers may be repeat participants and make numbers of new teachers a lower value.			We will offer spots to new teachers first.	T. Cartwright, Carver, Licwov-Channell, Wang
33	Insufficient recruitment of high school teachers, particularly new teachers in subsequent years of workshop.			Early recruitment/commitment of teacher involvement.	T. Cartwright, Carver, Licwov-Channell, Wang
34	For HS teachers, if the workshop is held at the same time across the 3 campuses, will there be adequate numbers of teachers participating (particularly at WVU & SU and WVSU & MU which are located in same region of WV)?			Early recruitment/commitment of teacher involvement.	T. Cartwright, Carver, Licwov-Channell, Wang
35	Brain Camp has a relatively low number of targeted students to participate in Year 2 might not meet a "critical mass" for a successful camp experience.			Instead of using the budget to pay students to attend, use that money to increase the number of supported students who can attend by providing them more food/materials during the workshop.	T. Cartwright (MU)
36	Brain Camp Student participants at MU will be only "day campers" because there is no funding to support them staying overnight on campus like at WVU.			Recruit local students who can return home at night.	T. Cartwright
37	UG Research Lack of interdepartmental communication (WVSU).			Create opportunities for mentors and faculty mentors to share research ideas.	Preterotto/ Bright
38	Internships The Shepherd coordinator has no previous experience working with neuroscience companies.			The coordinator will learn from online to get familiar with neuroscience companies and reach out to the HR office for potential partnership.	Wang

39	Decreasing participation in post-secondary education in general, and STEM in particular.			Communication, show the value to STEM education and research experience; make it relevant and relatable to students.	Strait
40	Brain Camp Well developed model at WVU may be resistant to new ideas from teachers.			Work with existing WVU Brain Camp personnel to add to current curriculum in meaningful ways.	Carver, Licwov-Channell (WVU)
Risk Category: Partnerships					
41	No Neuroscience companies in the state, yet.			Connect with companies outside the state and try and attract them; partner with other EPSCoR states that have industry.	Serafin
42	Barriers to data sharing.			Create data sharing rules in Collaboration Plan.	Strait

WV-NFNT Metrics and Outputs

Please see Table 1 for the number of K-12 students and teachers, undergraduate and graduate students and faculty impacted by the education, workforce development and diversity activities and involved in research programs.

	Y1	Y2	Y3	Y4	Y5
Theme 1					
Manuscripts submitted			1	2	6
Proposals submitted			1	5	1
Theme 2					
Manuscripts submitted			4	1	8
Proposals submitted			1	2	1
Faculty Hires		3			
EWDD					
Code WV lessons		5	5	5	5
Code WV Data Science Class					1
Brain Camp and Neuroscience activities and teacher PD shared on WV Dept of Ed platform		1	1	1	1
Structured mentoring modules for MU	1				
Structured mentoring modules for WVSU	1				
Internship Portal on HEPC website	1				
SU students participate summer research experiences at MU or WVU	2	2	2	2	2

Appendix 1: List of Participants

Project Leaders	Title	Institution	Role(s)
Juliana Serafin	Senior Director, Science, Technology & Research	West Virginia Higher Education Policy Commission	Project Director; Principal Investigator
Randy Nelson	Professor & Chair; Hazel Ruby McQuain Chair for Neurological Research	West Virginia University	Co-Principal Investigator
Umesh Reddy	Professor of Genetics and Genomics	West Virginia State University	Co-Principal Investigator
Nadja Spitzer	Associate Professor of Biological Sciences	Marshall University	Co-Principal Investigator, Theme 2 Lead
Suzanne Strait	Associate Director, Science, Technology & Research	West Virginia Higher Education Policy Commission	Co-Principal Investigator
Team Members	**Title**	**Institution**	**Role(s)**
Ariel (Aric) Agmon	Professor, Department of Neuroscience	West Virginia University	Research Theme 1
Vathani Amarashingham	4H STEM Specialist	West Virginia State University	EWDD
Charles Anderson	Assistant Professor, Department of Neuroscience	West Virginia University	Research Theme 2
Brian Antonsen	Biology Chair & Associate Professor	Marshall University	Project Management
Michelle Bridi	Assistant Professor, Department of Neuroscience	West Virginia University	Research Theme 1
Morgan Bridi	Assistant Professor, Department of Neuroscience	West Virginia University	Research Theme 1
Douglas Bright	EPSCoR Project Coordinator for Undergraduate Research	West Virginia State University	EWDD
David Cartwright	Research Associate, Computer and Information Technology	Marshall University	EWDD
Tina Cartwright	Professor; Curriculum, Instruction and Foundations	Marshall University	EWDD
Jeffrey Carver	Director of K-12 STEM Education Initiative and Associate Professor of Science Education	West Virginia University	EWDD
Michael Fultz	Associate Provost & Associate Vice President for Academic Affairs; Professor of Chemistry	West Virginia State University	Project Management
Brandon Henderson	Associate Professor, Department of Biomedical Sciences	Marshall University	Research Theme 2
Eric Horstick	Assistant Professor, Departments of Biology and Neuroscience	West Virginia University	Theme 1 Lead, WG2 Lead
Martin Hruska	Assistant Professor, Department of Neuroscience	West Virginia University	WG1 Lead
Michael Hu	Assistant Professor; Department of Microbiology, Immunology & Cell Biology	West Virginia University	Research Transcriptomics
Vanessa Licwov-Channel	STEM Service Assistant Professor	West Virginia University	EWDD
Ana Lima	Biomedical Research Scientist	Oregon Health Science Center	EAB
David Loomis	Evaluator	The Mark USA	Evaluation
Aamer Mahood	Director of the Shared Research Facilities	West Virginia University	Infrastructure
Haroon Malik	Assistant Professor, Department of Computer Science	Marshall University	Infrastructure
Josh Morganti	Assistant Professor, Department of Neuroscience	University of Kentucky	External Consultant

Sheena Murphy	Associate Vice President for Research Development	West Virginia University	Infrastructure
Purushothaman Natarajan	Associate Faculty Member, Biotechnology	West Virginia State University	Research Theme 1
Alejandro Nato	Assistant Professor, Department of Biomedical Sciences	Marshall University	Transcriptomics
Michael Norton	Professor, Department of Chemistry	Marshall University	Infrastructure
Ryan Percifield	WVU Genomics Core Director/Manager	West Virginia University	Transcriptomics
Graziele Preterotto	Graduate Coordinator	West Virginia State University	EWDD
Rosalynn Quiñones	Associate Professor, Department of Chemistry	Marshall University	EWDD
Chris Risher	Assistant Professor of Biomedical Sciences	Marshall University	Research Theme 2
Mary-Louise Risher	Assistant Professor of Biomedical Sciences	Marshall University	Research Theme 2
Colin Saldanha	Professor, Department of Neuroscience	American University	EAB
Barbara Shinn-Cunningham	Director, Carnegie Mellon Neuroscience Institute George A. and Helen Dunham Cowan Professor of Auditory Neuroscience, Biomedical Engineering, Psychology, and Electrical & Computer Engineering	Carnegie Mellon University	EAB
Conor Sipe	Assistant Professor, Department of Biology	Shepherd University	WG3 Lead Research Theme 1
Gay Stewart	Eberly Professor of STEM Education	West Virginia University	EWDD
Amber Stoffel	CodeWV Program Assistant	West Virginia University	EWDD
Qing Wang	Professor of Mathematics	Shepherd University	EWDD
Sijung Yun	Lecturer, Biotechnology	Johns Hopkins/Yotta Biomed	EAB
Hongkui Zeng	Executive Vice President, Director of the Allen Institute for Brain Science	Allen Institute for Brain Science	EAB

Appendix 2: Abbreviations and Acronyms

AC	Auditory Cortex
AgNP	Silver nanoparticles
CNS	Central Nervous System
E/I	Excitation / Inhibition
EAB	External Advisory Board
EELTR	Extended Executive Leadership Team for Resources
ELT	Executive Leadership Team
EPSCoR	Established Program to Stimulate Competitive Research
EWDD	Education, Workforce Development and Diversity
GEAR UP	Gaining Early Awareness and Readiness for Undergraduate Programs
HEPC	Higher Education Policy Commission
HS	High School
HSTA	Health Sciences and Technology Academy
IAB	Industry Advisory Board
IHC	Immunohistochemistry
INCLUDES	Inclusion across the Nation of Communities of Learners of Underrepresented Discoverers in Engineering and Science
LOF	Loss of Function
LSAMP	Louis Stokes Alliance for Minority Participation
MERFISH	Multiplexed, Error-Robust Fluorescent In-Situ Hybridization
MU	Marshall University
NSC	Neural Stem Cells
NSF	National Science Foundation
NURO	Neuroscience Undergraduate Research Opportunity
NYSF	National Youth Science Foundation
OFC	Orbital Frontal Cortex
P4E	Partnership for Equity
PBI	Project Based Instruction
PFC	Prefrontal Cortex
PTC	Programmatic Terms and Conditions
PVN-CRH	Paraventricular Nucleus - Corticotropin-Releasing Hormone
RNA	Ribonucleic Acid
S-STEM	Scholarships in STEM (see STEM)
STaR	Science, Technology and Research
STED	Stimulated Emission Depletion
STEM	Science, Technology, Engineering and Mathematics
SU	Shepherd University
TRiP-CRISPR	Transgenic RNAi Project - CRISPR
URG	Under-Represented Groups
URM	Under-Represented Minorities
WVSU	West Virginia State University
WVU	West Virginia University
WV-NFNT	West Virginia Network for Functional Neuroscience and Transcriptomics