



First Annual Symposium Center for Molecular Integrative Neuroresilience

Icahn School of Medicine at Mount Sinai

Program Director Giulio Maria Pasinetti

<http://icahn.mssm.edu/research/molecular-neuroresilience>

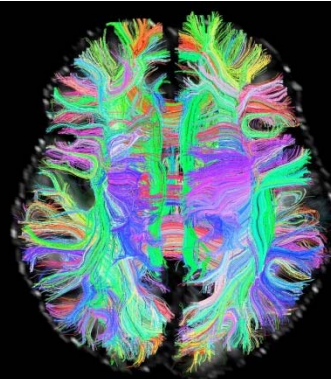
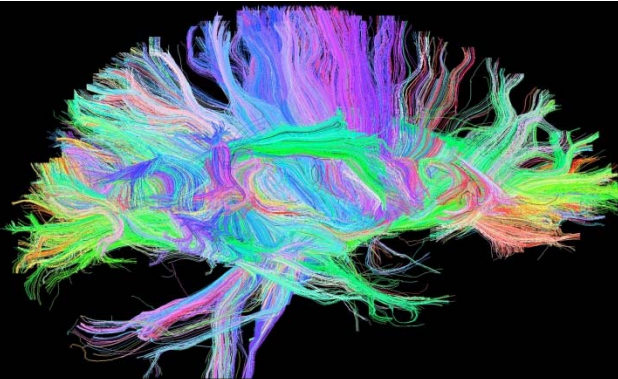
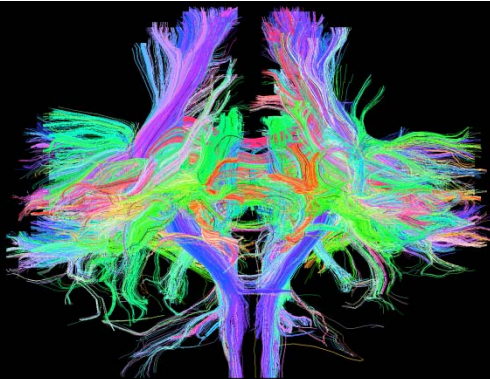
June 15th, 2016 from 9:00am – 5:00pm

New York Academy of Medicine, 1216 5th Ave, New York, NY 10029

Topics will include:

- Stress-induced psychological and cognitive impairment
- Mechanisms of neuroresilience and proteostasis
- Characterization of natural compounds for therapeutic and preventive interventions
- Role of the microbiome in promoting therapeutic efficacy

No registration costs • Free breakfast and lunch RSVP to libby.ward@mssm.edu by June 8th



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AGENDA OF SYMPOSIUM

Wednesday, June 15th, 2016

- 9:00am – 9:30am** **Registration and breakfast**
- 9:30am – 9:45am** **Introduction and overview of the Center**
Giulio Maria Pasinetti, MD, PhD
Saunders Family Chair and Professor of Neurology
- 9:45am – 10:15am** **Polyphenol compounds promote resilience to social stress (Project 1)**
Scott Russo, PhD
Associate Professor of Neuroscience
- 10:15am – 10:45am** **Molecular mechanisms associated with sleep deprivation-induced cognitive impairment (Project 2)**
Jun Wang, PhD
Assistant Professor of Neurology
- 10:45am – 11:00pm** **Break**
- 11:00am – 11:30am** **Exploring the interrelationships between diet, the microbiome, and the host immune system (Project 3)**
Jeremiah Faith, PhD
Assistant Professor of Clinical Immunology and of Genetics and Genomic Sciences
- 11:30am – 12:00pm** **Role of intestinal microbiota in the preservation of psychological and cognitive resilience (Project 3)**
Lap Ho, PhD
Associate Professor of Neurology
- 12:00pm – 1:00pm** **Lunch, poster presentations, and external advisory committee meeting**
- 1:00pm – 1:30pm** **Bioanalytical and biosynthetic approaches to the development of bioactive polyphenol metabolites to promote psychological and cognitive resilience (Core B)**
James Simon, PhD
Distinguished Professor of Plant Biology
- 1:30pm – 2:00pm** **Neurophysiological basis of resilience to social stress (Core C)**
Ming-Hu Han, PhD
Associate Professor of Neuroscience and of Pharmacology and Systems Therapeutics

2:00pm – 2:30pm	Managing, sharing and statistical modeling of multi-scale data: polyphenols, metabolites, phenotypic outcomes and beyond (Core A) Ke Hao, PhD Associate Professor of Genetics and Genomic Sciences
2:30pm – 2:45pm	Break
2:45pm – 3:15pm	Investigation of the role of BDNF/TrkB and VGF signaling in the antidepressant and pro-cognitive efficacy of grape-derived polyphenols (Pilot Research Program) Stephen Salton, PhD Professor of Neuroscience and of Geriatrics and Palliative Medicine
3:15pm – 4:00pm	Open discussion
4:00pm – 4:15pm	Conclusion of meeting and future plans

MISSION STATEMENT

The mission of the Center for Molecular integrative Neuroresilience is to provide a unique and detailed dissection of the role of dietary polyphenolic botanical supplements in the maintenance and promotion of psychological and cognitive resilience in models of social defeat and sleep deprivation.

COLLABORATING INSTITUTIONS

Icahn School of Medicine at Mount Sinai

James J. Peters VA Medical Center

Rutgers University

University of North Texas

ACKNOWLEDGMENTS

This symposium is the result of work supported by Grant Number P50 AT008661-01 entitled “Dietary Botanicals in the Preservation of Cognitive and Psychological Resilience” of the Centers for Advancing Research on Botanicals and Other Natural Products (CARBON) Program from the National Center for Complementary and Integrative Health (NCCIH) and the Office of Dietary Supplements (ODS).

OVERVIEW OF RESEARCH PROJECTS AND CORES

A. SIGNIFICANCE

The Center for Molecular Integrative Neuroresilience at the Icahn School of Medicine at Mount Sinai (ISMMS), in response to the funding opportunity RFA –OD-14-001 from the National Center for Complementary and Alternative Medicine (NCCAM) and the Office of Dietary Supplements (ODS), is a joint effort by investigators from: the Departments of Neurology, Neuroscience, Medicine, and Immunology at ISMMS; the Department of Biological Sciences at the University of North Texas (UNT); the Agriculture and Plant Biology Department at Rutgers, State University of New Jersey (Rutgers); USDA Agricultural Research Services at Beltsville; the Department of Food Biology, Purdue University; the Nutrition and Food Science Department at Texas A&M University; and the Botanical Technology Development Center at the University of Illinois Chicago (UIC). We define a vision for an integrated, multidisciplinary program of preclinical research projects all linked by a unifying scientific theme related to characterizing the Role of Dietary Botanicals in the Preservation of Cognitive and Psychological Resilience.

Resilience to psychological and physiological stress:

Over the past decade, there has been increasing attention paid to the phenomenon of resilience: the ability to maintain normal psychological and physical functioning and avoid serious mental illness when exposed to stress and trauma, even at extraordinary levels. Although resilience has been identified across the spectrum of psychiatric disorders, we focus here on resilience as it relates to post-traumatic stress disorder (PTSD) and major depressive disorder (MDD) (**Project 1**) and acute/chronic sleep deprivation (**Project 2**). In this context, resilience refers to the capacity of an individual to cope with negative psychological and biological consequences of extreme stress that would otherwise compromise their psychological or physical wellbeing. Recent reports indicate that resilience in humans is an active, adaptive process and not simply the absence of pathological responses that occur in more susceptible individuals. Anxiety and stress-related disorders are widespread psychological conditions with broad health implications, including negative impacts on cardiovascular and metabolic functions as well as on mental health, including depression and memory dysfunction. The concept of resilience is difficult to operationalize, as it encapsulates many divergent behavioral phenotypes. Indeed, the study of human resilience is still a mostly phenomenological assessment of biological factors in resilient individuals that are associated with more successful coping responses.

Mechanisms underlying resilience to social defeat and sleep deprivation-mediated cognitive decline:

Social defeat in humans, as in chronic social subordination in mice, leads to a spectrum of depression-like behaviors (1). Social defeat may lead to a social avoidance state, also known as “anhedonia,” in a subset of mice termed “susceptible,” whereas another subset of “resilient mice” resists the development of such behaviors (2). We have strong evidence suggesting that increased peripheral proinflammatory cytokine production is an important factor in the development of depression or anxiety (3-6). In a mouse model of repeated social defeat stress (RSDS), we found that by reducing peripheral inflammation, such as reducing the content of the cytokine Interleukin-6 (IL-6) released from leukocytes, we can promote psychological resilience and brain health (3-5). Little is known about the mechanisms associated with cognitive resilience in models of sleep deprivation (SD) and no remedies have been discovered to promote resilience and to maintain learning and memory functions (7). Acute/Chronic SD or sleep loss contributes to stress and impaired psychological vigilance (8), and is a widespread problem in our society (9). According to the Center

for Disease Control and Prevention, 50-70 million individuals in the US alone report inadequate sleep (10), and the negative impacts of SD on physical and cognitive health strain our healthcare system (11). Mice with experimental SD exhibit memory impairment, as assessed by classical Morris water maze and fear condition testing (12), which is associated with perturbation of molecular signaling underlying learning and memory functions (13). We and others found that SD down-regulates the cAMP response element-binding protein (CREB) (14), a transcription factor downstream of cAMP/PKA signaling pathway; decreases transcription of plasticity-related genes (15) and inhibits the induction of LTP (16). Therefore, targeting molecular mechanisms associated with the negative consequences of social defeat or sleep deprivation may present novel strategy for promoting resiliencies.

Dietary botanical supplements in stress: implications in the promotion of resilience:

Extensive evidence documents that physiological stress has a detrimental impact on psychological health, cognitive functions, and ultimately wellbeing. While stressful events are an important cause of psychopathology, most individuals exposed to adversity maintain normal psychological functioning. The mechanisms underlying resilience are poorly understood, and there is no successful approach to maintain or promote resilience under stressful events. Natural dietary supplements have been used to treat stress and insomnia since Hippocrates, and often have only mild side effects that are easily managed (6;17). Naturally occurring polyphenols have been shown to promote health through a broad range of mechanisms; most excitingly and relevant to the studies, we note that grape derived flavan-3-ols have extensive pharmacological action, including anti-inflammatory properties, and exert antidepressant-like activities in animal models (18;19). Most excitingly, in our feasibility studies supporting the Center, we found: (1) dietary supplementation with a combination of grape seed polyphenol extract (GSPE), Concord grape juice (CGJ) and resveratrol (RSV) (herein referred to as the botanical supplement mixture) promotes psychological resilience in the mouse model of RSDS, in part, through modulation of peripheral inflammation within leukocytes (e.g. reduce IL-6 production) and prevention of maladaptive synaptic plasticity in the brain (**Project 1**) (2) Oral administration with botanical supplement mixture attenuates SD-mediated memory impairments assessed by contextual fear conditioning assay and the improvement of memory consolidation process coincides with the induction of the cAMP-response element binding (CREB) transcription factor activity and increased gene expression of proteins involved in the consolidation of short into long term memory in the brain of the treated group, relative to the untreated controls following acute SD (**Project 2**).

Role of microbiome in polyphenol bioavailability and cognitive and psychological resilience:

The polyphenols in botanical supplement mixture are extensively metabolized and absorbed following oral consumption. Due to the diverse susceptibility of phenolic compounds to metabolism by gut enzymes and GI microbiota, their bioavailability can vary from very low to very high (20). Our pilot feasibility evidence revealed an approximately 10-folds reduction in the content of 3,4-dihydroxycinnamic acid and approximately 50% reduction in content of 3-(3'-hydroxyphenyl) propionic acid and homovanillic acid in plasma of germ-free gnotobiotic mice compared to conventionalized gnotobiotic mice (with normal microbiota harvested from a wild-type mice) following oral administration of the botanical supplement mixture (**Project 3**). Our evidence highlights contributions the GI microbiota in modulating bioconversion and bioavailability of bioactive phenolic acids from the botanical mixture relevant for preservation of psychological resilience. Based on these data, we hypothesize that a select panel of biologically available phenolic metabolites, upon reaching the circulatory system and the brain, may modulate cellular and

molecular mechanisms to reduce inflammatory activities in the periphery and to promote neuroplasticity following exposure to stress such as social defeat or SD (Figure 1). Therefore, the overall goals of the studies in the Center are: (i) To mechanistically investigate the beneficial effect of the above botanical supplements in promoting resilience against psychological and physiological stress (ii) To identify microbiota that are critical for the generation of bioactive phenolic metabolites related to resilience and to develop next-generation probiotics capable of promoting bioavailability of the bioactive phenolic metabolites to enhance the efficacy of these botanical supplements in preserving psychological and cognitive resilience.

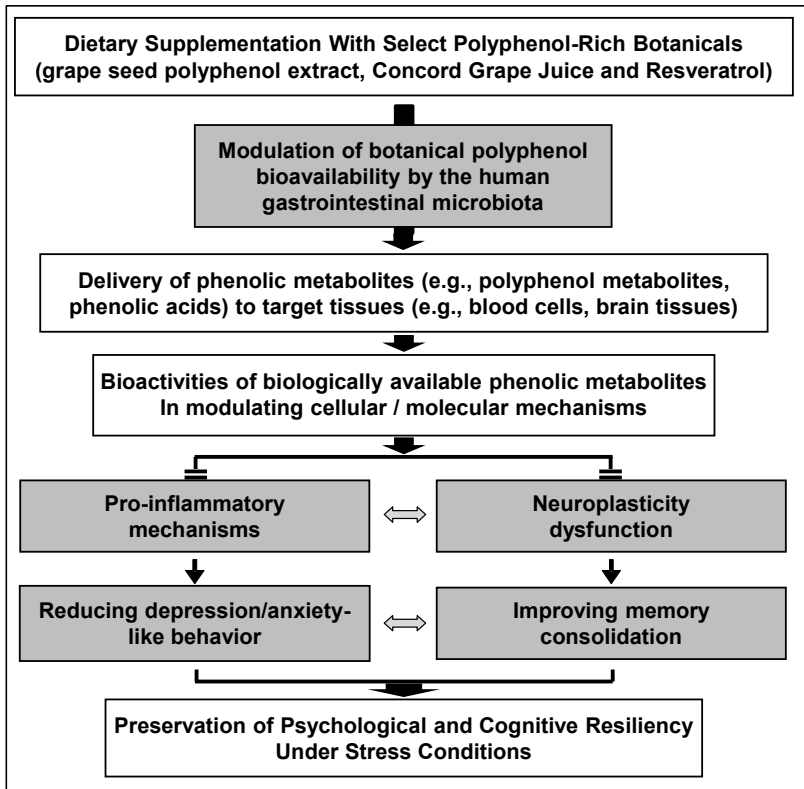


Fig. 1. Overall working hypothesis for the efficacy of dietary supplementation with botanicals in preserving cognitive and psychological resilience and to generate next-generation probiotics to promote the utilization of botanical supplements.

B. INNOVATION

The studies are innovative with regard to *concept, scope, and technological development*. The studies are conceptually innovative because, for the first time, we are conducting a comparative *in vitro* and *in vivo* assessment of the role of dietary bioavailable botanical supplements in modulating psychological and cognitive resilience in lieu of acute and chronic stress. While there has been significant effort within the pharmaceutical and medical industries to develop treatments for mood disorders such as anxiety and

depression, currently available treatments often have extreme side effects, adverse drug reactions, as well as inconvenient drug-drug and food-drug interactions. Moreover, thirty percent of patients fail to respond to currently available therapies and 70% never achieve complete remission (21). Thus, our mechanistic studies, by challenging the prevailing and failing approach of the pharmaceutical industry and demonstrating the role of a novel botanical supplement, are conceptually innovative and in line with the current dogma for much needed advances in psychological health (22). As designed, our study will provide, for the first time, mechanistic information supporting the potential application of certain botanical supplements to promote cognitive and psychological resilience. Our study is also innovative in terms of scope, since it will fill the fundamental gaps for future translational studies by providing novel strategies to synthetically generate the bioavailable phenolic metabolites for mechanistic and bioactivity studies. Moreover, the studies to characterize subset(s) of GI microbiota strain(s) (**Project 3**) critical for generation of specific bioactive phenolic metabolites that contribute to cognitive wellness and psychological resilience is highly innovative and will provide insight into broader issues related to the translational goal of the study. Lastly, we note that the studies in the Research Projects were designed as a unique multiscale technological innovative approach, made possible by our interdisciplinary research team with outstanding expertise in

psychiatry, neurology, neuroscience, pharmacology, microbiology, food science, botany and chemistry (phytochemistry and analytical chemistry). For example, Project 3 is assisting Project 1 in the re-derivation of transgenic mice expressing GFP under the D1 or D2 promoter as germ-free, which is extremely innovative, reflects the novel technology of application, and the synergistic nature of the studies' design. Consistent with the NIH BRAIN Initiative, in the Center, we are employing newly developed technologies, including epigenetics, optogenetics and gnotobiotics, for our investigation to allow for revolutionary and dynamic insight into brain wellness and mechanisms promoting cognitive and psychological resilience.

C. APPROACH

C.1. Working hypothesis

Based on our previous studies, we hypothesized that select phenolic metabolites derived from botanical supplements may modulate mechanisms underlying resilient vs. susceptible phenotype in the models of chronic social stress and sleep deprivation, therefore, the overall goal of our Center is to investigate the cellular and molecular mechanisms by which botanical supplements may beneficially modulate to promote psychological (Project 1) and cognitive (Project 2) resilience in the context of chronic social stress and sleep deprivation. We are also testing whether supplementation of next-generation probiotics can improve the bioactivity of the botanical supplements (Project 3). The working hypothesis for each project is illustrated in Fig. 2 (please refer to individual projects for details).

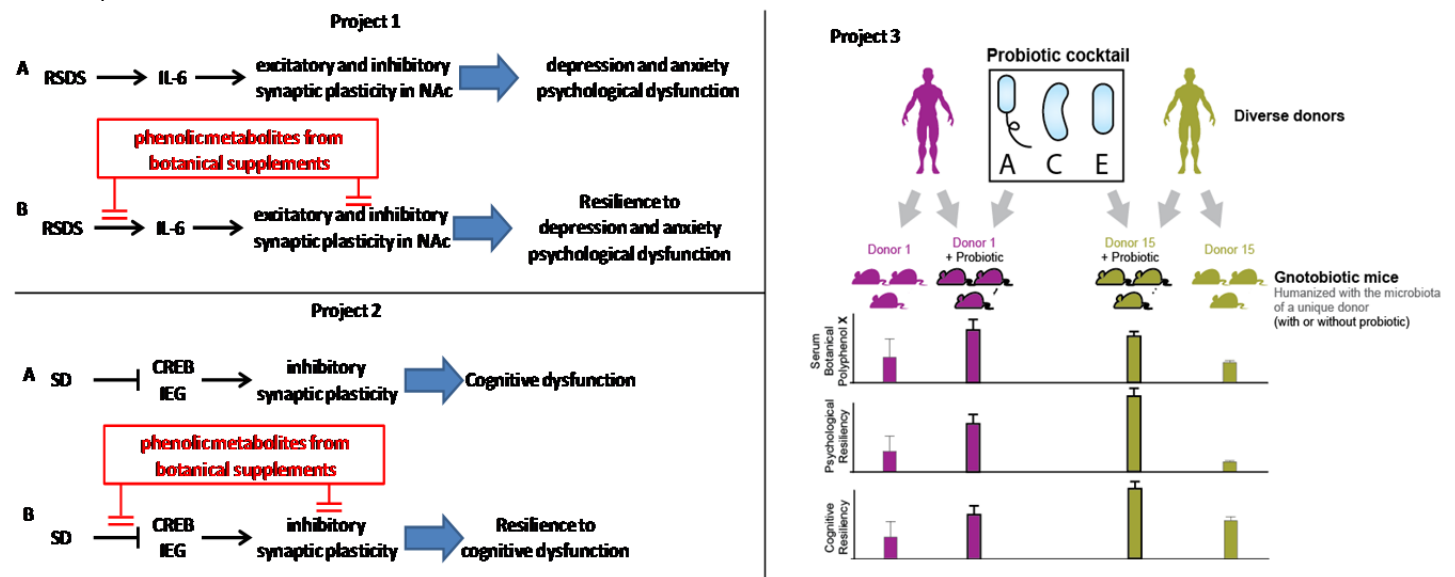


Fig. 2. Working hypothesis for Projects 1, 2 and 3. **Project 1:** Phenolic metabolites derived from dietary botanical supplements (i) reduce RSDS-induced inflammation (ii) directly attenuate synaptic plasticity change, which will lead to resilience to psychological dysfunction; **Project 2:** Phenolic metabolites (i) promote cAMP response element-binding protein (CREB) signaling (ii) modulate the expression of immediate early genes (IEGs) that are absolutely necessary for memory consolidation will lead to resilience to sleep deprivation-mediated cognitive dysfunction; **Project 3:** There are significant quantitative differences between gut microbiota of rodents and humans. We hypothesize these differences will modulate generation of biologically available, bioactive phenolic metabolites, particularly phenolic acids, affecting the efficacy of the botanical mixture in promoting psychological and/or cognitive resilience in response to stress challenges. We also hypothesize that next-generation probiotics designed to promote bioconversion of these select bioactive phenolic acids will enhance the efficacy of the botanical supplement mixture.

There is an unprecedented consumer demand for foods or food constituents that help prevent or manage health conditions, including conditions associated with psychological stress such as poor concentration, depression, and anxiety (21;22). These botanical supplements present an invaluable potential solution as they are relatively inexpensive, have few perceived side effects, and are non-invasive compared to other neurological and psychiatric treatments. Moreover, and relevant to the overall goal of NCCAM and the ODS, the studies are allowing a mechanistic understanding of the effects of select bioactive phenolic metabolites in the promotion of psychological and cognitive resilience.

C.2. Characterization of botanical supplement mixture and phenolic metabolites

Select grape-derived dietary botanical supplement mixture, specifically, GSPE, CGJ and RSV, exhibit a wide range of physiological activities. Primary phenolic constituents of grape and grape-derived products include proanthocyanidins (PACs), their flavan-3-ol precursors, and anthocyanidins(AC) (23), as well as flavonols, phenolic acids, and the stilbene resveratrol (Fig. 3.). Previous phytochemical and bio-guided fractionation studies from our research team revealed that GSPE is rich in catechin (C), epicatechin (EC), PAC oligomers, and gallic acid, and CGJ (and wine) is rich in ACs, PACs, and flavonols along with some phenolic acids (24-26) (see list of publications following the References list in this section).

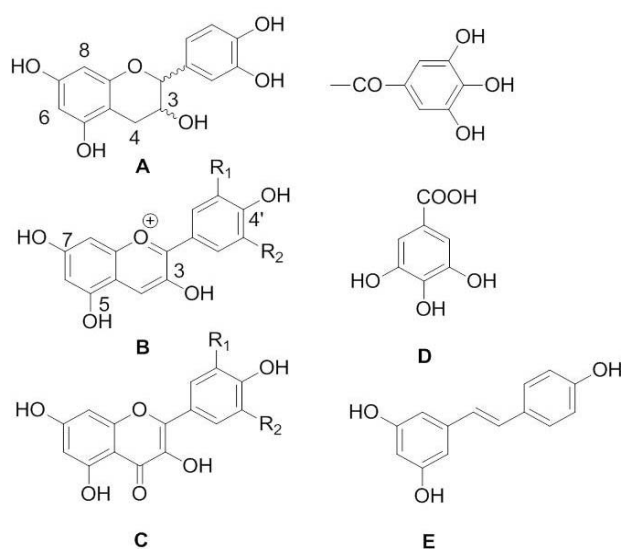


Fig. 3. Representative structures of polyphenols from grape and grape-derived products.

A) General structure of grape PACs: the main units of the PACs are C, EC, and epicatechin gallate (ECG). These are linked to each other by either C4-C8 or C4-C6 bonds, and gallate is generally substituted for H on the hydroxyl at position 3. **B)** General structure of AC: R=H/OH/OMe; the glycosides are termed ACs. 3 and/or 5 glycosides are commonly available in grape. **C)** General structure of grape flavonols: R=H/OH/OMe; 3, 5, 7 and/or 4' glycosides are commonly available; **D)** Gallic acid. **E)** RSV.

Previous studies by our research group demonstrated that oral administration of the botanical supplement mixture are effective in protecting against

neuropathology and cognitive impairment in neurodegenerative disorders such as Alzheimer's disease and tau-mediated neurodegenerative disorder. These studies, supported by a National Institute of Health-National Center for Complementary and Alternative Medicine (NCCAM)-Funded Center on dietary polyphenols in AD treatment as potential novel agents in AD prevention/treatment (funded period 2007 – 2014), were extremely successful, as reflected by 39 publications from the funded period 2007-2014 (Appendix 1). Among significant findings from our NCCAM-funded studies are the identification of 18 biologically available phenolic metabolites, including 16 polyphenol metabolites (27;28)(Pasinetti, unpublished observation) and 2 phenolic acids (Wang and Pasinetti, unpublished observation) that are found accumulated in the brain (Table I), with the potential to protect against AD pathogenic mechanisms in the brain. Moreover, in ongoing studies, we have demonstrated some of these brain-accumulating polyphenol metabolites, in particular, 3'-OMe-epicatechin-5-glucuronide (25), quercetin-glucuronide (24), as well as 3-hydroxybenzoic acid and 3-(3'-hydroxyphenyl) propionic acid (see Research Project 3 for details) are capable of contributing to

the efficacy of these botanical supplements to interfere with the mechanisms associated with cognitive and psychology resilience.

Of particular interest to our Center, we recently observed that dietary supplementation with the botanical supplement mixture significantly improved preservation of psychological and cognitive resilience under challenge by stress conditions in experimental mouse models (please see Research Projects 1 and 2 for details). In addition to the above-mentioned 16 polyphenol metabolites and 2 phenolic acids that are able to target the brain, studies from our group and from others also identified 8 more biologically available phenolic acids from either the GSPE (Wang and Pasinetti, unpublished); Ho and Faith, unpublished) or the GCJ (29-33) component of the botanical mixture that are found accumulated in blood (Table I). The collective panel of 26 biologically available phenolic metabolites (16 polyphenol metabolites and 10 phenolic acids) from the botanical mixture is listed in Table I. Based on this, our Center is designed to clarify the mechanisms by individual biologically available

					phenolic compounds
biologically available	urine	plasma	brain	commercial	for delivering
phenolic meabolites	detection	detection	detection	availability	phenolic metabolites
polyphenol metabolites					
catechin-5-glucuronide	in progress	+ ^a	+ ^a	no	catechin
3'-OME-catechin-5-glucuronide	in progress	+ ^a	+ ^a	no	catechin
epicatechin-5-glucuronide	in progress	+ ^a	+ ^a	no	epicatechin
3'-OME-epicatechin-5-glucuronide	in progress	+ ^a	+ ^a	no	epicatechin
quercetin-glucuronide	in progress	+ ^b	+ ^b	yes	quercetin
OMe-quercetin-glucuronide	in progress	+ ^b	+ ^b	no	quercetin
malvidin-3-glucoside	in progress	+ ^b	+ ^b	yes	malvidin-glucoside
malvidin-glucuronide	in progress	+ ^b	+ ^b	no	malvidin-glucoside
cyanidin-3-glucoside	in progress	+ ^b	+ ^b	yes	cyanidin3-glucoside
cyanidin-glucuronide	in progress	+ ^b	+ ^b	no	cyanidin3-glucoside
delphinidin-3-glucoside	in progress	+ ^b	+ ^b	yes	delphinidin-3-glucoside
delphinidin-glucuronide	in progress	+ ^b	+ ^b	no	delphinidin-3-glucoside
peonidin-3-glucoside	in progress	+ ^b	+ ^b	yes	peonidin-3-glucoside
resveratrol	in progress	+ ^c	+ ^c	yes	resveratrol
resveratrol-glucuronide	in progress	+ ^c	+ ^c	yes	resveratrol
Ome-resveratrol-glucuronide	in progress	+ ^c	+ ^c	no	resveratrol
Phenolic acids					
3-hydroxybenzoic acid	+ ^d	- ^d	+ ^d	yes	3-hydroxybenzoic acid
3-(3'-hydroxyphenyl) propionic acid *	+ ^d	+ ^d	+ ^d	yes	3-(3'-hydroxyphenyl) propionic acid
hippuric acid	+ ^d	+ ^d	in progress	yes	hippuric acid
5-(4'-hydroxyphenyl)valeric acid	+ ^d	+ ^d	in progress	yes	5-(4'-hydroxyphenyl)valeric acid
homovanillic acid *	in progress	+ ^e	in progress	yes	homovanillic acid
3,4-dihydroxydrocinnamic acid *	+ ^f	+ ^e	in progress	yes	3,4-dihydroxydrocinnamic acid
3,4 dihydroxyphenylacetic acid	+ ^f	+ ^G	in progress	yes	3,4 dihydroxyphenylacetic acid
vanillic acid	+ ^h	+ ⁱ	in progress	yes	vanillic acid
p-coumaric acid	+ ^j	+ ^j	in progress	yes	p-coumaric acid
dihydrocoumaric acid	+ ^j	+ ^j	in progress	no	dihydrocoumaric acid

phenolic metabolites from this panel contribute to the benefits of the botanical mixture in preserving psychological and cognitive resilience by modulating specific cellular/molecular processes.

Table 1: Biologically available phenolic metabolites and corresponding phenolic compounds for in vivo delivery to be investigated in our Center. Listed are 26 phenolic metabolites (16 polyphenol metabolites and 10 phenolic acids) from the botanical supplement mixture that are biologically available in the periphery and in the brain following oral administration of GSPE, CGJ or resveratrol. These biologically available phenolic metabolites are the focus of our Center. Provided are information

on detection in urine, plasma and brain. We also noted that some of these phenolic metabolites are commercially available. Those that are not commercially available are being generated biosynthetically (by Scientific Core B) for in vitro bioactivity and mechanistic studies. We also provide

a listed of commercially available phenolic compounds for the delivery of biologically bioavailable, bioactive phenolic metabolites to the periphery and/or the brain in our in vivo mechanistic studies, as we describe in more detail below. Upper case letters refer to sources of the information regarding urine, blood and brain detection: a, (27); b, Pasinetti, unpublished data; c,(28) ; d, Pasinetti and Wang, unpublished data; e, Ho and Faith, unpublished data; f, (30); g,(32) ; h,(33) ; i,(31) ; j, (29).

Research Projects 1 and 2 are specifically designed to characterize the cellular/molecular mechanism by individual phenolic metabolites in Table I contribute to the efficacy of the botanical mixture to modulate, respectively, psychology and cognitive resilience. As we discussed in more details in Project 1, our studies are supported by our feasibility evidence that specific biologically available phenolic acid in Table I, can contribute to the benefits of the botanical supplement mixture in preserving psychological resilience by modulating, inflammatory processes (e.g. 3,4 dihydroxyphenylacetic acid) and expression of synaptic genes (e.g., by 3-(3'-hydroxyphenyl) propionic acid and 3,4-dihydroxyhydrocinnamic acid) in the nucleus accumbens of the brain. Similarly, as we discussed in more details in Project 2, our studies are also supported by evidence that select phenolic metabolites from Table I can contribute to the efficacy of the botanical mixture to promote cognitive resilience by modulating neuronal synaptic plasticity (e.g., by the polyphenol metabolite, 3'-OMe-epicatechin-5-glucuronide and quercetin-glucuronide) as well as c-Fos, Arc, Erg cellular signaling pathways (e.g., by the phenolic acid, homovanillic acid and 3,4 dihydroxyphenylacetic acid).

Published evidence (34-37) has demonstrated that gut microbial community (i.e., gut microbiota) plays a critical role in modulating bioconversion and bioavailability of bioactive phenolic metabolites, particularly phenolic acids, from orally consumed polyphenol-rich botanical supplements. Moreover, as discussed in more details in Research Project 3, We observed an approximately 10-folds reduction in the content of 3,4-dihydroxyhydrocinnamic acid and approximately 50% reduction in content of 3-(3'-hydroxyphenyl) propionic acid and homovanillic acid in plasma of gnotobiotic mice compared to conventionalized gnotobiotic mice following oral administration of the botanical mixture (see Research Project 3 for more details). Our evidence highlights contributions the GI microbiota in modulating bioconversion and bioavailability of bioactive phenolic acids from the botanical mixture relevant for preservation of psychological resilience. Thus, the studies in Research Project 3 are designed to use "humanized" gnotobiotic mice, i.e., germ-free mice that are re-colonized with the microbiota of adult human donors, as a model system to identify human gut bacterial strain(s) that are critical for the generation of bioactive phenolic acids and for developing next-generation probiotics capable of promoting bioavailability of the bioactive botanical supplements to enhance the efficacy of these botanical supplements in preserving psychological and cognitive resilience. Validating specific probiotic strategies for promoting bioavailability of bioactive botanicals are providing an initial tool for experimental modulation of botanical bioavailability in animal models of psychological stress by Projects 1 and 2.

Potential capacity of biologically available phenolic metabolites to target cellular processes. Most phenolic compounds are too hydrophilic to penetrate cellular lipid bilayers by passive diffusion, and member carriers are required to transport biologically available phenolic metabolites across the gastrointestinal track, the blood-brain barrier (BBB), and cellular plasma membrane. While studies exploring transport of phenolic metabolites are outside of the scope of our Center, there is already information on membrane carriers that could be involved in absorption/transport of phenolic compounds, particularly across the BBB and cellular membrane. Indeed, recent studies have demonstrated involvement of certain transporters, such as the sodium-dependent glucose transporter and the organic anion transporter 3, in transporting select phenolic compounds across BBB endothelial cells (38). The capability of certain phenolic metabolites to penetrate the BBB and

subsequently the cellular plasma membrane of brain cells is consistent with observations from us and others (26;27;39) that select phenolic compounds are capable of accumulating in brain tissues and some brain-bioavailable phenolic compounds are capable of modulating cellular processes, such as CREB signaling, as we describe in more detail in Research Projects 2.

We note that some of the phenolic metabolites in Table I are glucuronide derivatives. The addition of a glucuronide moiety to a xenobiotic such as a phenolic compound increases its hydrophilicity, which typically is thought to promote transport of the phenolic compound out of the cells for eventual excretion from the body. Nonetheless, our findings of specific steady-state accumulation of select phenolic glucuronide compounds in brain tissues suggest the potential for these compounds to exert bioactivities in the brain. Thus, our Center is investigating the potential value of all 26 phenolic metabolites from Table I for preservation of psychological and cognitive resilience as we proposed in Projects 1 and 2.

Sources of phenolic metabolites for in vitro studies Research Projects 1 and 2 are using in vitro model systems and assess the 26 biologically bioavailable phenolic metabolites listed in Table for bioactivities targeted by the Research Project, followed by mechanistic studies and in vivo efficacy testing. Majority of the phenolic metabolites listed in Table 1 can be obtained commercially. However, a number of polyphenol metabolites in Table I are not commercially available and these are being generated by our innovative polyphenol biosynthesis approach developed by Core B as schematically depicted in Fig. 4, which is capable of producing microgram quantity of individual phenolic metabolites for in vitro studies. Thus, Research Projects 1 and 2 are affording for the first time access to unique biologically available, bioactive phenolic metabolites for extensive bioactivity and mechanistic studies. Core B has already successfully bio-synthesized brain-bioavailable catechin glucuronides, one epicatechin glucuronide and one methyl-catechin glucuronide (not shown), which we identified in the brain following oral administration of polyphenol precursor, and we have shown that yeast microsomes expressing human UGT1A9 are an optimal means to synthesize glucuronation of epicatechin and other polyphenols) for the execution of Projects 1 and 2. (This provides a facile route to synthesize several of the polyphenol metabolites highlighted in Table 1 for *in vitro* bioactivity and mechanistic studies.

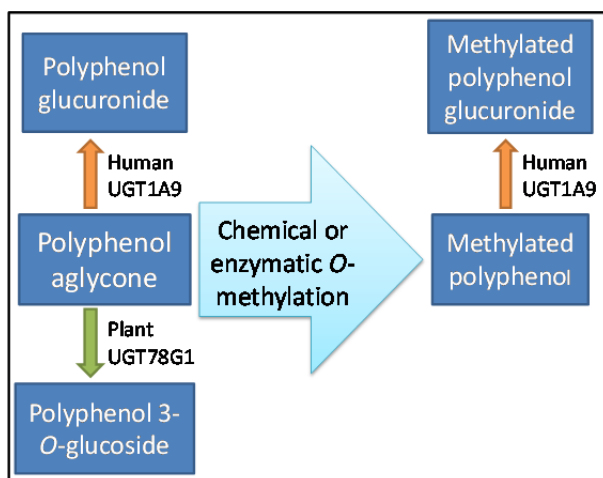


Fig. 4. Biosynthetic approaches to generate biologically available, bioactive phenolic metabolites utilizing a combination of chemical (methylation) and enzymatic (glycosylation) derivations of commercially available precursors. Abbreviations: UGT, UDP-glucuronosyltransferase.

Novel strategy using commercially available polyphenol compounds as a means to deliver select bioactive phenolic metabolites to peripheral and brain tissues in experimental rodent models. Many polyphenols from botanicals are extensively metabolized during intestinal absorption and post-absorptive xenobiotic metabolism

(24;40). This raises the issue of how best to deliver bioactive polyphenol metabolites from Table I to peripheral and/or brain target tissues in mouse models. While there is limited understanding of polyphenol metabolism in mammals, we have previously demonstrated that oral administration of certain brain-bioavailable phenolic glucoside, such as malvidin-3-glucoside, cyaniding-3-glucoside, delphinidin-3-glucoside and peonidin-3-glucoside, as well as resveratrol, listed in Table I, result in

their intact delivery to the brain (41). However, there is no information on specific phenolic compounds that can be used to deliver targeted phenolic glucuronides, listed in Table I, to the brain. Since mammalian cells express glucuronosyltransferases that catalyze glucuronation of polyphenols and other small molecules, we conducted a feasibility study to test whether the use of phenolic aglycones (phenolic compounds in the absence of the attachment of sugar groups) could generate and deliver specific phenolic glucuronide metabolites, listed in Table I, to the brain. We treated Sprague-Dawley rats with a mixture of commercially available phenolic aglycones in Table I for ten days, to simulate chronic treatment, and assessed the content of phenolic metabolites in the perfused brain. We found that orally administered select phenolic compounds leads to the accumulation of the corresponding phenolic metabolites at μM and sub- μM in the brain (Fig. 5). Based on this, Research Projects 1 and 2 are conducting a series of dose-finding studies to identify the most appropriate efficacious dose-range of using select phenolic compounds to deliver corresponding bioactive polyphenol metabolites for engaging with peripheral and/or central pathways relevant to psychological and/or cognitive resilience in specific experimental models. Previous evidence suggests that oral doses in humans, equivalent to a mouse dose up to 240 mg/kg/BW/day for individual polyphenol precursor listed in Table I, are safe and tolerable for long-term treatment (42-44). We started with doses surrounding 240 mg/kg/BW/day for bioavailability dose finding studies.

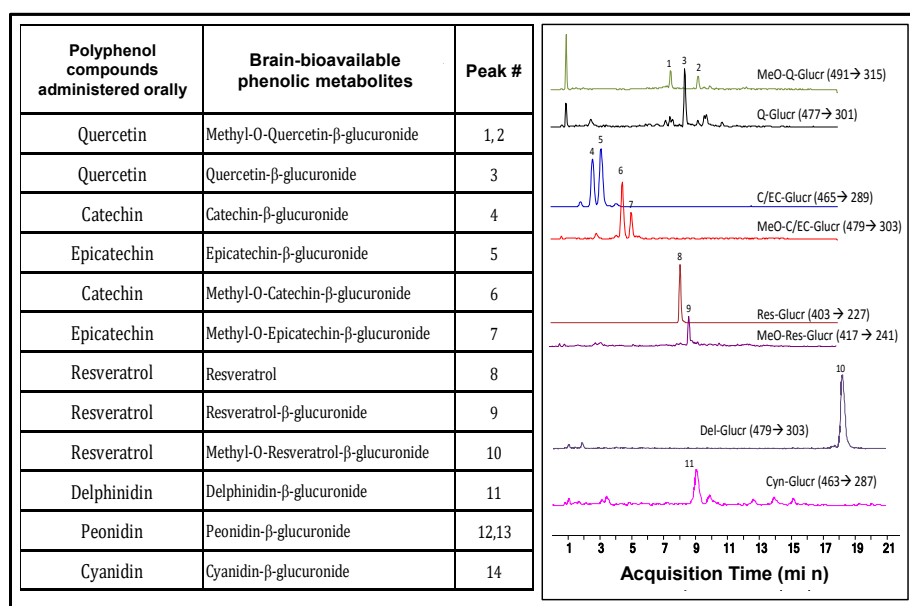


Fig. 5. Identification of brain-bioavailable phenol glucuronide metabolites in perfused rat brains following sub-acute treatment with the mixture of phenolic aglycone compounds. Rats were treated by gavage with the mixture of phenolic aglycone compounds at a dose equivalent to a mouse dose of 300 mg/Kg-BW/day for 10 days and perfused with PBS. The contents of polyphenol metabolites in perfused brain specimens were analyzed by LC-MS/MS. Left panel, phenolic glucuronide compounds detected in brain specimens, n=3 per group; right panel, Representative LC-MS/MS spectra of individual brain-bioavailable phenolic glucuronide compounds.

right panel, Representative LC-MS/MS spectra of individual brain-bioavailable phenolic glucuronide compounds.

There is limited information on how best to deliver phenolic acids to target tissues. Since bioconversion of botanical polyphenol components to phenolic acids occur in the colon followed by absorption by colonocyte. Base on this, we are delivering individual phenolic acids from Table I by orally administrating animals with the same phenolic acid, as depicted in Table I. While orally administered phenolic acid may undergo structural modification or degradation in the GI, we anticipate some of phenolic acid will reach the colon for absorption. Consistent with this approach, Lafay et al. 2006 orally administered rats with the phenolic acid, chlorogenic acid and observed accumulation of intact chlorogenic acid (as well as caffeic acid, a hydrolytic metabolite of chlorogenic acid) in plasma(45). We note that in humans, daily total consumption of polyphenols and phenolic

acids has been estimated at 1 g/day, with phenolic acids accounting for approximately 30% of the total intake, or 330 mg/day (46). Calculating equivalent dose between species based on body surface using a USDA recommended formula, a human dose of 330 mg/day is equivalent to 62 mg/ Kg-BW/day in mice. Thus, for our in vivo studies, we are using phenolic doses surrounding 62 mg/ Kg-BW /day. We note that Alam et al. 2013 treated hypertensive rats with ferulic acid at 50 mg/ Kg-BW/day (equivalent to a mouse dose of 98 mg/ Kg-BW /day) and reported improved cardiovascular and kidney structure/function in the absence of adverse effect(47). Therefore, we are considering testing doses higher than 63 mg/Kg-BW/day for specific phenolic acids from Table I for in vivo studies, when necessary.

C.2. Overall Center structure and research design

Our studies are designed to investigate the cellular and molecular mechanisms by which dietary supplementation with specific botanical supplement mixture, namely GSPE, CGJ, and RSV, may help preserve psychological and cognitive resilience under conditions of psychological and physiological stress induced by social defeat and sleep deprivation. To accomplish this goal, we have designed three Research Projects (**Research Projects 1- 3**) and three supportive Research Cores (**Core A, B, C**), as we schematically depict in Fig. 6 below. Research **Projects 1** and **2** are designed to investigate the mechanisms by which select biologically available phenolic metabolites from GSPE, CGJ and RSV may modulate specific mechanisms leading to the preservation of psychological (**Project 1**) and cognitive (**Project 2**) resilience under stress conditions. Research **Project 3** is investigating “combinatorial microbe communities in humanized gnotobiotic mice” to better define specific bacterial strains common in healthy humans that are critical for the bioavailability of the specific phenolic metabolites. Investigations by all Research Projects are supported by the three Research Cores.

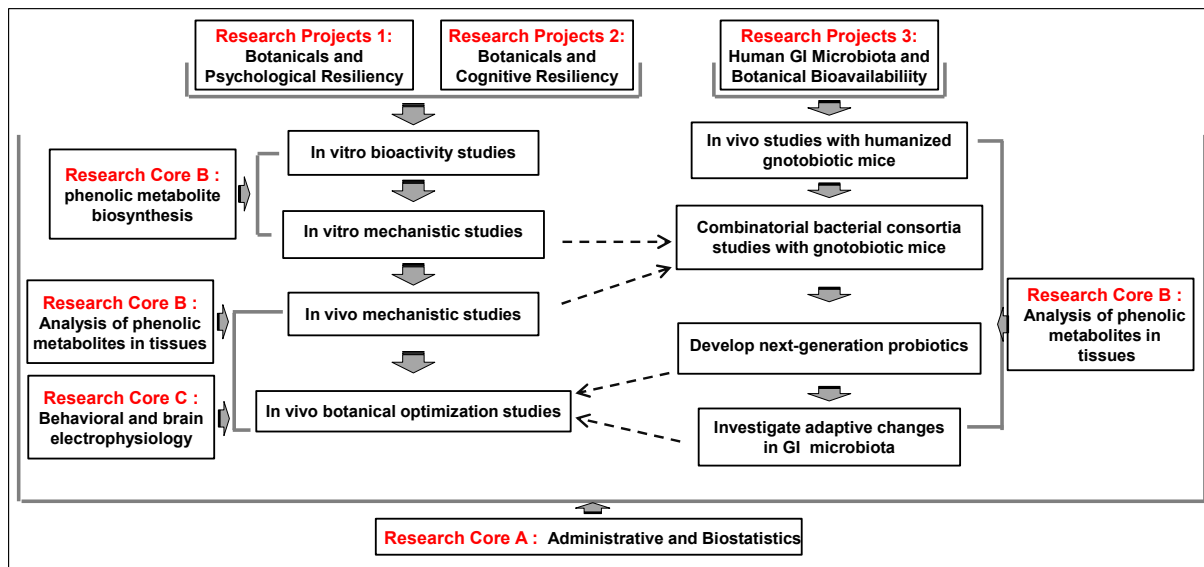


Fig. 6. Structural organization of the studies This scheme illustrates the studies by Research Projects 1, 2 and 3 and identifies key points of interactions among the

Research Projects, Research Cores A, B and C that are critical for the successful execution of the studies.

Research Projects 1 and 2 are operating as follows, and as schematically illustrated in Fig. 6:

- First conduct a comprehensive battery of in vitro bioactivity and mechanistic studies to assess each phenolic metabolite, from our established panel (Table 1), for their bioactivities in modulating key physiological mechanisms contributing to psychological or cognitive resilience.

Projects 1 and 2 each rank orders and selects the top two metabolites with the most robust *in vitro* bioactivities for *in vivo* testing

- Following the *in vitro* survey, conduct *in vivo* efficacy and mechanistic studies, based on dietary supplementation with specific phenolic compound (Table 1) to deliver specific phenolic metabolites to target tissues, to assess their effectiveness and mechanisms identified from *in vitro* studies.
- Conduct *in vivo* botanical optimization studies to explore the feasibility of developing a next-generation probiotic to promote the efficacy of GSPE, CGJ, and RSV in preserving psychological and cognitive resilience under conditions of social defeat and sleep deprivation

As discussed above, human GI microbiota play a key role in dictating the bioavailability of dietary botanical supplements as a function of age, inflammatory conditions, certain dietary regimens, and certain disease conditions (48;49). In initial studies, Research Project 3 is conducting a series of *in vivo* studies with humanized gnotobiotic mice to explore delivery and accumulation of botanical phenolic metabolites in the context of human microbiota, and subsequently conduct combinatorial bacterial consortia studies with gnotobiotic mice to identify bacterial strains critical for generation of individual (or subsets) of bioavailable, bioactive phenolic metabolites from GSPE, CGF and RSV. Research Project 3, in close conjunction with Projects 1 and 2, operates as follows, and as schematically depicted in Fig. 6:

- Conduct studies using botanicals without specific polyphenol components in gnotobiotic mice to identify individual (or subsets of) phenolic components in the botanical that are critical for the generation of specific biologically available, bioactive phenolic metabolites.
- Based on information gathered from the identification of specific bacterial strains contributing to the generation of physiologically relevant phenolic metabolites, continue to use the gnotobiotic mouse model to explore the development of a next-generation probiotic that can be used to modulate bioavailability of individual or subsets of bioavailable, bioactive phenolic metabolites from GSPE, CGJ, and RSV.
- Investigate adaptive changes in GI microbiota to identify potential effects of polyphenol-rich diets, SD, and social defeat in modulating GI bacterial strains critical for generation of bioavailable bioactive phenolic metabolites from GSPE, CGJ, and RSV that would affect the impacts of these botanicals in the preservation of psychological and/or cognitive resilience.

The support from the three Research Cores is critical for the successful execution of individual Research Projects. As illustrated in Fig. 4, the **Administrative/Statistical Core A** provides oversight/coordination, in addition to Biostatistical and Educational/Training support, for the Center activities. The **Biosynthetic Component of Core B** generates phenolic metabolites to support all *in vitro bioactivity and mechanistic studies* by **Research Projects 1 and 2**. Moreover, the **Bioanalytical Component of Core B** monitors the bioavailability of phenolic metabolites in gnotobiotic mice following treatment with GSPE, CGJ, and RSV for Research Project 3 studies. Moreover, the **Bioanalytical Component of Core B** provides critical support for **Research Projects 1 and 2** by monitoring the bioavailability of specific bioactive phenolic metabolites following treatments with phenolic compound in the *in vivo* mechanistic studies, as well as following treatments with GSPE, CGJ, and RSV in the *in vivo botanical optimization studies*. The **Behavioral and Electrophysiology Core C** operates by providing behavioral phenotyping support for all the studies in Research Projects 1-2, and measures mini excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) studies in Project 1 and LTP studies in Project 2.

C.3. Interactions and synergy among Research Projects and Cores

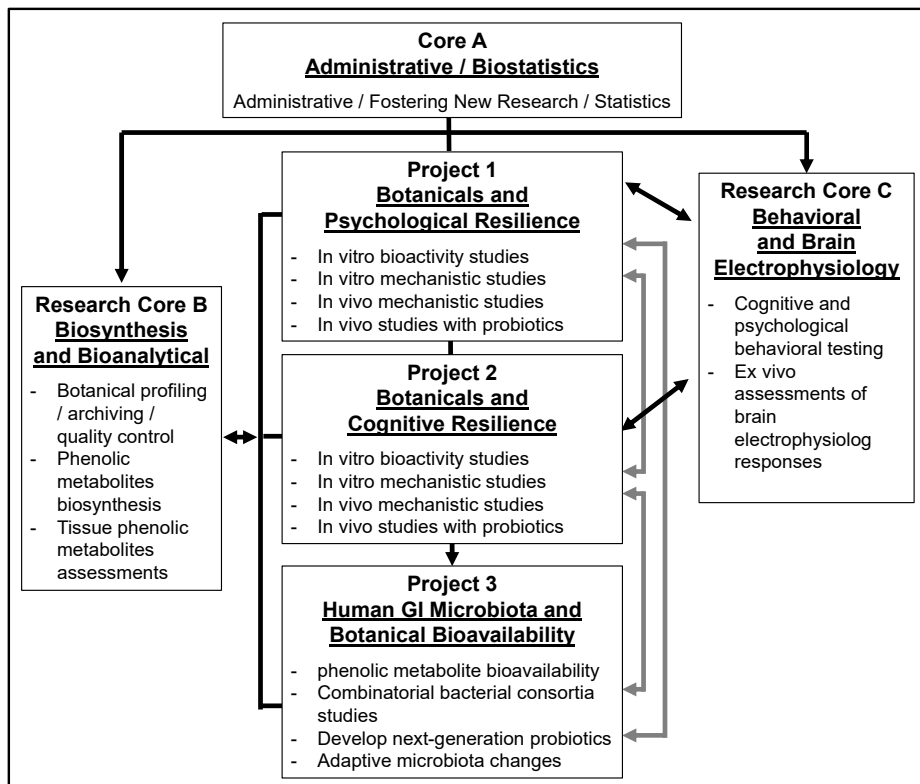


Fig. 7 Interactions between Research Projects and Cores within the Botanical Center.

This Center is specifically designed to maximize collaboration and scientific synergy among all Research Projects and Cores. These interactions are schematically depicted in Fig. 7. **Projects 1 and 2** conduct *in vitro* studies to assess each phenolic metabolite, from our established panel of bioavailable phenolic metabolites (Table 1), for their bioactivities in modulating key mechanisms contributing to cognitive or psychological resilience, respectively. They then select the metabolites with the most robust bioactivities for *in vivo*, preclinical studies to validate that delivery of these metabolites to target tissues (e.g., inflammatory blood cells and brain tissues) lead to preservation of cognitive resilience under conditions of

SD or preservation of psychological resilience under conditions of social defeat. These *in vitro* bioactivity and mechanistic studies are supported by the **Core B Biosynthetic Component**, which is responsible for generating the phenolic metabolites. Moreover, in collaboration with the **Core B Bioanalytical Component**, **Projects 1 and 2** conduct *in vivo* studies using polyphenol found in the botanicals to deliver specific bioactive phenolic metabolite(s) to target tissues (e.g., blood cells and brain tissue) to validate the physiological relevance of these metabolites in modulating mechanisms identified in *in vitro* studies. Moreover, in collaboration with **Research Project 3**, **Projects 1 and 2** are testing the efficacy of next-generation probiotics, designed to promote bioavailability of specific bioactive phenolic metabolites, to enhance the benefits of these botanicals in preserving psychological and cognitive resilience. These botanical efficacy studies are supported by **Core C Behavioral Phenotyping and Electrophysiology Components**, to assess the impact of botanical treatment, in the presence or absence of probiotics, on reducing psychological behavioral responses and/or improving cognitive, behavioral, and brain neuroplasticity responses underlying memory consolidation. Interactions with **Projects 1 and 2** help **Project 3** prioritize its investigation to identify bacterial strains(s) capable generating individual (or panels of) phenolic metabolites that are effective in modulating mechanisms underlying preservation of psychological and/or cognitive resilience, and all of the **Project 3** studies are conducted in close collaboration with the **Core B Bioanalytical Component**, to determine bioavailability of GSPE/CGJ/RSV phenolic metabolites in gnotobiotic mice, in the absence or presence of human microbiota or subsets of bacterial strains. As further discussed in the independent research proposals, there is explicit synergy between **Projects 1 and 2** due to evidence suggesting certain mechanisms, including regulation of synaptic plasticity gene expression,

are common in response to social stress and SD. The Research Projects also synergistically interact through the built-in collaboration between **Research Project 3** and **Projects 1 and 2**, to prioritize bacteria strain(s) critical for the generation of phenolic metabolites effective in promoting the preservation of psychological and/or cognitive resilience. This interaction allows **Projects 1 and 2** to identify the most appropriate next-generation probiotic that might be effective in enhancing the efficacy of GSPE, CGJ, and RSV in preserving psychological and cognitive resilience. To maximize efficiency and streamline the daily operations of this Center, **Core A** is specifically structured in committee and working groups designed to monitor efficiency, streamline interactions, resolve disputes, etc. between the Center's investigators.

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EXTERNAL ADVISORY COMMITTEE MEMBERS

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Preservation of Psychological Resilience Under Chronic Stress (Project 1)

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Georgia Hodes	Icahn School of Medicine at Mount Sinai
Caroline Menard	Icahn School of Medicine at Mount Sinai
Lena Khibnik	Icahn School of Medicine at Mount Sinai

Promotion of Resilience Against Cognitive Decline Induced by Sleep Deprivation (Project 2)

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Chad Smith	Icahn School of Medicine at Mount Sinai
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Tom Villani	Rutgers University
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Icahn School of Medicine at Mount Sinai
University of Pennsylvania
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BIOGRAPHIES OF PROJECT AND CORE LEADERS

Giulio Maria Pasinetti, MD, PhD

Program Director, Core A Leader, & Project 2 Leader

Dr. Pasinetti is the Saunders Family Chair and Chief of Neurodiagnostics and Neurotherapeutics in the Friedman Brain Institute in the Department of Neurology at Mount Sinai with a broad background in age-related neurological disorders. He has more than 20 years of experience in experimental neurodegeneration and preclinical aging, and has been the recipient of more than 40 grants, including funding from the National Institutes of Health for the identification of early biological markers of Alzheimer's disease (AD) dementia and a Phase II clinical grant for AD. He is also the director of a NIH/NCCAM funded center on dietary polyphenols in AD. He has a strong record of successful and productive research endeavors exploring the role of polyphenols in modulating mechanisms associated with neurodegenerative disorders, as well in promoting cellular and molecular processes. In the last 10 years, Dr. Pasinetti has been a pioneer in the development of studies related to polyphenol compounds as potential novel therapeutic agents that may engage with AD pathogenic pathways. Collectively, Dr. Pasinetti's strong record of achievement, in the direction of the NIH/NCCAM funded Center as well as more than 20 years of experience in the prevention of neurodegeneration, promotion of brain health, and preclinical research in animal models of disease, provide testimonial for the continued success of the Center for Molecular Integrative Neuroresilience.

Richard A. Dixon, PhD

Co-Program Director & Core B Leader

Dr. Dixon is a Distinguished Research Professor in the Department of Biological Sciences at the University of North Texas. His research interests center on the biosynthesis, molecular biology and metabolic engineering of plant natural product pathways, with specific emphasis on flavonoids and lignins. Dr. Dixon has published over 430 papers on these and related topics in international journals, and have been named by the Institute for Scientific Information as one of the 10 most cited authors in the plant and animal sciences. He is a Fellow of the American Association for the Advancement of Science, and was elected to membership of the US National Academy of Sciences in 2007. He has particular expertise in the biosynthesis of polyphenols, including condensed tannins, in plants, and has been a member of Dr. Pasinetti's NCCAM-funded CERC team for the past six years, in which capacity his group has provided defined size-fractionated grape seed polyphenolic fractions and developed biochemical synthetic approaches to generate the bioactive metabolites of grape seed polyphenols that are found in the brain and may be involved in long term memory potentiation. His group is providing this synthetic capacity for the generation of unlabeled and radiolabeled flavonoid metabolites for use as standards and for mechanism of action studies in Projects 1 and 2 of the Center.

Scott J Russo, PhD

Project 1 Leader

Dr. Russo is an Assistant Professor of Neuroscience at the Icahn School of Medicine at Mount Sinai. A major focus of his research is to study the neural and immunological mechanisms of neuropsychiatric disorders such as depression and anxiety. He uses a combination of transgenic mice, immune cell transplantation, optogenetics/electrophysiology, viral mediated gene transfer, behavioral models and molecular methods to understand how the brain adapts to stress or drugs to control pathological behaviors in depression and anxiety. Over the past 10 years, Dr. Russo has been very active in the mental health and drug addiction fields, evidenced by a number of recent honors;

he was named a Kavli National Academy of Science Frontiers fellow in 2009, elected to the Faculty of 1000 in 2012, awarded an individual NIMH R01 award in 2010, elected a full member of the American College of Neuropsychopharmacology in 2011, and received a Johnson and Johnson/IMHRO Rising Star Translational Research Award in 2011. Dr. Russo has published over 60 peer-reviewed manuscripts in the past decade within the fields of stress and addiction neurobiology.

Jun Wang, PhD

Project 1 Leader & Core C Leader

Dr. Wang is an Assistant Professor of Neurology at the Icahn School of Medicine at Mount Sinai. Dr. Wang has been leading the Behavioral Phenotyping Core for the NIH/NCCAM funded research on dietary polyphenols in AD since 2007 and has extensive experience with animal behavior studies. She has been instrumental in selecting and establishing a versatile set of behavioral tests that can efficiently characterize those core behavioral domains of high interest to cognitive function. The Core successfully completed all proposed studies and participated in the publication of over 10 scientific papers involving the utilization of the Core. Dr. Wang has extensive knowledge of grape-derived botanicals, including their absorption, metabolism, brain accumulation, and bioactivities in the brain. She has carried out many efficacy as well as mechanistic studies to identify specific botanical compounds that might interfere with disease mechanisms (including those pertinent to AD, Huntington's disease, and type II diabetes mellitus). Some of the compounds identified have successfully progressed to clinical trials. Collectively, Dr. Wang's expertise and prior work make her well suited to successfully lead Project 1 and Core C of the Center.

Jeremiah James Faith, PhD

Project 3 Leader

Dr. Faith is an Assistant Professor of Clinical Immunology and of Genetics and Genomic Sciences at the Icahn School of Medicine at Mount Sinai. His research focuses on data driven computational modeling of the microbiome with predictions that are followed-up experimentally with new or existing technologies. He has pursued aspects of this paradigm for the past 13 years, first as a scientific programmer studying evolutionary genomics, then as a graduate student developing network inference algorithms for large-scale bacterial microarray datasets, and as a postdoctoral fellow and instructor in Dr. Jeffrey Gordon's lab at Washington University in St. Louis where he applied statistical modeling and systems biology tools to the field of gut microbiome research. He has over 6 years of experience working in the microbiome field, developing improvements in the scale and quality of 16S rRNA amplicon sequencing, and optimized rRNA depletion protocols and data processing pipelines for RNA-Seq metatranscriptomics of both the human and the mouse microbiota. Of particular relevance for this Center are the anaerobic robotics pipeline he developed to isolate a large proportion of the gut microbes from human stool or intestinal contents combined with expertise in systems biology and gnotobiotics to enable the systematic identification of gut microbial strains that modulate aspects of host physiology. The nutritional content of our diets is dependent not only on the chemical composition of the dietary ingredients consumed but also on unique set of microbes each individual harbors in their intestine. In the last five years, he has developed tools to understand the interactions among diet, the gut microbiota, and host physiology. This Center seamlessly follows Dr. Faith's prior research and current interests as it combines his interest in identifying microbial strains that modulate host physiology with his interest in the interrelationship among host diet, gut microbes, and health. The technologies developed and provided by the core components of this program project provide well-established methods to track these polyphenols throughout the body. The uniqueness of these polyphenols combined with our ability to provide polyphenol-free diets to animal models

provides a unique opportunity to study the dynamics of these bioactive compounds throughout the body in the context of different gut microbial communities.

Lap Ho, PhD

Project 3 Leader

Dr. Ho is an Associate Professor of Neurology at the Icahn School of Medicine at Mount Sinai. He is trained in nutrition, biochemistry and molecular biology, with more than 20 years of experience in translational neuroscience research in AD and other neurodegenerative disorders. His work has generated insights on the molecular mechanisms underlying the onset and progression of neurodegenerative disorders, and he has translated this information into multiple therapeutic developments. To date, his research has resulted in 80 papers, published in peer-reviewed journals. He has extensive experience with experimental and preclinical studies characterizing the bioactivities of polyphenol components in the brain and the beneficial impacts of dietary supplementation with polyphenol preparations in reducing AD mechanisms in mouse models. He has extensive knowledge and expertise in polyphenols and their metabolism, and the design and execution of preclinical and clinical studies exploring potential health benefits of polyphenol-rich botanical dietary supplements.

Ke Hao, PhD

Core A Leader

Dr. Hao is an Associate Professor of Genetics and Genomic Sciences at the Icahn School of Medicine at Mount Sinai. His research field is statistical genetics and epidemiology, with specific goals to identify the genetic basis underlying human diseases and interactions with environmental exposures. In the past decade, he has been actively involved in the fast evolution of the field and made significant contributions to the field. In the biotechnology arena, he developed the Affymetrix SNP 5.0 and 6.0 microarrays, which are now mainstream tools in genetic studies. Further, he characterized the impacts of measurement error and informative missing. Such works provided valuable guidance on detecting and correcting for confounders. On statistical methodology, he developed a variety of algorithms widely used by researchers, including multi-SNP tagging and HGPD-eigen. In gene mapping, he led and conducted a large number of genetics studies, including linkage scans, candidate gene approaches, and genome-wide association studies (GWAS), on many disease areas (e.g. asthma, cancer, preterm delivery, obesity, CHD, etc). In recent years, he initiated and led large scale next generation sequencing (NGS) applications on whole-genome, whole-transcriptome and meta-genome levels, the somatic mutation and structure variations were linked to clinical outcomes (e.g. cancer prognosis). He also has invested significant effort in the downstream mining (ie, results interpretation) of genetic studies. He created large expression QTL (eQTL) data sets on many human tissue types (e.g., lung, liver, blood, adipose, brain, etc.), and characterized the architecture of the genetic control of gene expression as well as gene networks.

James E. Simon, PhD

Core B Leader

Dr. Simon is a Professor of Plant Biology and Member of the Graduate Faculty in Plant Biology and Medicinal Chemistry at Rutgers University. His research is focused on natural products chemistry, plant genetics and biology, botanical authentication, standardization and quality control. He was the founder of and continue to serve as the Director of the Rutgers New Use Agriculture and Natural Plant Products Program (NUANPP), which identifies new bioactive compounds in plants, finds new applications of bioactive compounds from fruits, vegetables, botanicals and develops standardized botanical extracts and products for health and nutrition. He has published over 250 scientific papers,

authored and edited over 10 scientific books and holds several patents including a patent on geraniol synthase (US patent no. US 7,704,716 B2); and others on use of plants and extracts for medicinal applications (e.g. Peperidine-flavan alkaloid compounds derived from African herb tea kinkeliba as anti-diabetic agents. US Patent No. US 8,642,769 B2; Antioxidant and anti-inflammatory activity of compounds and preparations from African nutmeg seeds. US Patent No. 7,371,413 B2). As a Professor at Purdue University for 17 years and Rutgers University for 14 years, his research involves extensive botanical collection, controlled grow-outs and genetic and chemical profiling to examine the genetic and chemodiversity of species. His research also focuses on botanical authentication using classical botanical taxonomy, genetics and chemical fingerprinting, biological studies, quality control and the recurring issues associated with adulteration of botanicals.

Ming-Hu Han, PhD

Core C Leader

Dr. Han is an Associate Professor of Neuroscience and of Pharmacology and Systems Therapeutics. He received his doctoral training in Dr. Xiong-Li Yang's lab at the Chinese Academy of Sciences, completed his postdoctoral research in Dr. Colin Barnstable's lab at Penn State, and learned brain slice recording techniques in Dr. George Aghajanian's lab at Yale University School of Medicine. The extensive training he received at these laboratories led to his mastery of state-of-the-art electrophysiological techniques. Dr. Han later joined Dr. Eric Nestler's research group at the University of Texas Southwestern Medical Center in Dallas as a junior faculty member. With his neurophysiology background, he independently established an electrophysiology lab in that group and developed a novel, powerful slice culture system to study neuronal intrinsic properties by using viral-mediated gene transfer; a system that has been successfully implemented in many publications (*J Neurosci*, 2006; *Cell*, 2007; *JBC*, 2008; *J Neurosci*, 2010; *PNAS*, 2010; *Neuropsychopharmacology* 2011; *J Neurosci*, 2011; *Neuron*, 2011; *Nat Neurosci*, 2012). Moreover, through collaborations and extensive research experiences in the molecular and behavioral research groups, he learned to effectively integrate various complementary methodologies in order to gain novel insight into a biological phenomenon with direct relevance to neuropsychiatry. For more advanced integrative techniques, he learned optogenetic approaches from Dr. Karl Deisseroth's research group at Stanford University and produced some interesting physiological and behavioral data by manipulating nucleus accumbens neurons (*Science*, 2010) and ventral tegmental area dopamine neurons (*Science*, 2012; *Nature*, 2013; *Nat Neurosci*, 2014; *Science*, 2014) in freely behaving animals. Collectively, his broad training and research experience, across molecular, cellular and behavioral levels, fully equipped him to lead the electrophysiology component of Research Core C in the Center.

PROGRESS REPORT OVERALL CENTER ACTIVITIES – YEAR 1

Project Title: Pasinetti: Overall; Dietary Botanicals in the Preservation of Cognitive and Psychological Resilience (Pasinetti)	
Grant Number: 5P50AT008661-02	Project/Grant Period: 09/01/2015 – 06/30/2020
Reporting Period: 09/01/2015 – 06/30/2016	Requested Budget Period: 07/01/2016 – 0/20/2017
Report Term Frequency: Annual	Date Submitted: 05/20/2016
Program Director/Principal Investigator Information: Giulio Maria Pasinetti, MD, PhD Phone number: (212) 241-7938 Email: giulio.pasinetti@mssm.edu	Recipient Organization: Icahn School of Medicine at Mount Sinai 1 Gustave L. Levy PI New York, NY 10029 DUNS: 078861598 EIN: 1136171197A1 Recipient ID: 14-1000-00001-01-PD
Administrative Official: Michael J. King Icahn School of Medicine at Mount Sinai 1 Gustave L. Levy PI New York, NY 10029 Phone Number: (212) 824-8300 Email: Michael.king@mssm.edu	Signing Official: Michael J. King Icahn School of Medicine at Mount Sinai 1 Gustave L. Levy PI New York, NY 10029 Phone Number: (212) 824-8300 Email: Michael.king@mssm.edu
Human Subjects: No	Vertebrate Animals: Yes
hESC: No	Inventions/Patents: Yes If yes, previously reported: No

OVERALL CENTER ACTIVITIES – YEAR 1

A. Specific Aims

The Specific Aims of the Overall Proposal have not changed from those originally proposed.

Research Project 1: Preservation of psychological resilience under chronic stress

Research Project 2: Promotion of resilience against cognitive decline induced by sleep deprivation

Research Project 3: Role of the microbiome in promotion of cognitive and psychological resilience

Research Cores: The execution of Research Projects 1-3 will be supported by three Research Cores: The **Administrative-Biostatistics/Data Management Core A** will provide administrative oversight to all components (Projects and Cores) of the overall BDSRC, as well as guidance on all issues related to study design and data entry, storage, analysis, and interpretation. The **Biosynthesis/Bioanalytical Core B** will support Research Projects 1- 3 by centralizing the procurement, synthesis, and quality assurance of dietary polyphenol botanical supplements, and by profiling the polyphenol compounds in target tissues. The **Behavioral Phenotyping and Electrophysiology Core C** will provide behavioral phenotyping services for all the studies proposed in Research Projects 1-3. Moreover, it will assist and execute excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) studies in Project 1 and long term potentiation (LTP) studies in Project 2.

B. Studies and Results

The overall goal of the Icahn School of Medicine at Mount Sinai (ISMMS) Botanical Dietary Supplement Research Center (BDSRC) is to provide a mechanistic understanding of the **role of grape derived dietary botanical supplements in the preservation and promotion of cognitive and psychological resilience** under certain stress conditions. Investigations by the ISMMS BDSRC have been conducted following the guidelines and timeline of the originally proposed studies, and significant progress has been made in the 9.5 month interval between initiation of funding on September 01, 2015 and the current submission of the Year 1 Progress Report. For the upcoming Year 2, the overall goal and Specific Aims of the ISMMS BDSRC as well as the timeline of the investigatory studies remain unchanged from those originally proposed.

Below, we summarized in brief the overall structure of the ISMMS BDSRC and overall goals of the individual BDSRC Research Projects and Scientific Cores. This will be followed by Progress Reports from individual Research Projects and Research Cores, which will provide more detail of progress made in Year 1 and projected Year 2 investigations by individual ISMMS BDSRC components.

Our BDSRC has been conceptualized and constructed based on recent evidence from our research team, which demonstrated that dietary supplementation with a specific grape-based Bioactive Dietary Polyphenol Preparation (BDPP), comprised of a grape seed polyphenol extract (GSPE), Concord grape juice (CGJ), and resveratrol (RSV), is highly effective in promoting resilience against the induction of cognitive impairment and/or depression by certain environmental stresses in experimental animal models. Based on extensive additional supportive feasibility evidence, we hypothesized that these cognitive and psychological health benefits are mediated by the action(s) of select polyphenol BDPP polyphenol components that, followed by oral BDPP administration, are biologically available in blood and/or target tissues and are biologically active in modulating specific pathogenic processes underlying the development of cognitive impairment and/or depression. Thus, our BDSRC is specifically designed to identify and structurally characterize specific biologically available, bioactive BDPP polyphenol components that may contribute to the efficacy of BDPP to

promote resilience to environmentally-induced cognitive dysfunction and/or depression, and to clarify the mechanisms underlying the cognitive and/or psychological health benefits of these bioactive polyphenol components. The ISSMS BDSRC is comprised of three Research Projects. Each of the Research Projects has specifically defined goals and objectives and investigations by individual Research Projects are facilitated by built-in interactions among the Research Projects with support of three Scientific Cores. Specific goals of individual Research Projects and Scientific Cores, interactions among Research Projects/Research Cores, progress made in Year 1 and projected investigations for Year 2 are as follows:

Research Project 1: Preservation of psychological resilience under chronic stress

The overall objective of Project 1 is to investigate the molecular and cellular mechanisms underlying the beneficial effects of dietary supplementation with BDPP in promoting psychological resiliency against chronic stress, in particular repeated social defeat stress (RSDS). Specifically, Project 1 will explore the role of individual BDPP-derived phenolic metabolites in modulating inflammatory responses in the periphery and their effect on stress-induced synaptic restructuring and plasticity in the central nervous system. Moreover, in collaboration with Project 3, Project 1 will use gnotobiotic mice to explore whether probiotic intervention with specific bacterial strains can enhance the efficacy of botanical supplements to render greater resilience in the context of RSDS. Year 1 investigations by Project 1 have validated two RSDS-mediated pathologic phenotypes – induction of IL-6 expression in the periphery and inhibition of Rac1 in the nucleus accumbens (NAc) in the brain – as key targets for intervention. Project 1 has screened a number of BDPP-derived phenolic metabolites and identified a number of phenolic compounds that are biologically available in circulating blood and/or the brain and are effective in protecting against molecular pathologic pathways underlying IL-6 induction and Rac1 inhibition. Moreover, Project 1 has shown that simultaneously targeting both IL-6 and Rac1 using a combination of two of these bioactive phenolic compounds – malvidin-glucoside and 3,4-dihydrocaffeic acid (DHCA) – significantly promoted resilience to RSDS-mediated psychological dysfunction. Outcomes from Project 1 studies during Year 1 provide the first experimental evidence that select botanical components can promote resilience to stress-mediated psychological disorders in part, by modulating multiple key pathologic mechanisms which are not addressed by classical antidepressants. This provides a proof of concept and experimental evidence that botanical supplements can be used to target novel mechanisms to preserve and to promote psychological resilience under stressful conditions.

Research Project 2: Promotion of resilience against cognitive decline induced by sleep deprivation

The overall objective of Project 2 is to explore the potential role of BDPP-derived brain bioavailable phenolic metabolites in mechanisms associated with synaptic plasticity and memory consolidation in the context of sleep deprivation (SD). In particular, Project 2 will investigate the effect of individual metabolites in protecting against SD-mediated disruptions in CREB and mTOR signaling, long-term potentiation (LTP) and immediate early gene (IEG) expression. Furthermore, in collaboration with Project 3, Project 2 will test the potential role of “next generation” probiotics in maximizing the effect of dietary polyphenol botanical supplements on promoting cognitive resilience. Year 1 investigations by Project 2 have screened a number of BDPP-derived phenolic metabolites and identified two biologically available phenolic metabolites, Quercetin-glucuronide (Quer-Gluc) and Malvidin-glucoside (Mal-Gluc), which are effective in protecting against SD-mediated disruptions in CREB and mTOR signaling, *in vitro*. Project 2 also clarified the molecular mechanisms of actions of these bioactive phenolic metabolites. Moreover, Project 2 has confirmed that oral administration of Quer-Gluc

precursor quercetin and Mal-Gluc significantly promoted resilience to cognitive function in a SD mouse model. Outcomes from these preclinical studies have provided a basis for potential translational applications of select botanicals in promoting resilience to SD-mediated cognitive dysfunction by simultaneously targeting multiple SD-mediated pathological processes.

Research Project 3: Role of the microbiome in promotion of cognitive and psychological resilience

The overall objective of Project 3 is to identify select bacterial strain(s) from human GI microbiota that are critical for the generation of BDPP-derived, biologically available, bioactive metabolites that are important for Projects 1 and 2 for the promotion of psychological and cognitive resilience. Using “humanized” gnotobiotic mice, Project 3 aims to develop novel “next-generation” probiotics for promoting the bioavailability of select bioactive phenolic metabolites, thereby enhancing the efficacy of BDPP (and other polyphenol-rich botanicals) in preserving psychological and cognitive resilience. Interactions with Projects 1-2 will help Project 3 prioritize its investigations to identify bacterial strains(s) capable of promoting the generation of individual (or panels) of phenolic metabolites that are effective in modulating mechanisms underlying preservation of psychological and/or cognitive resilience. During Year 1, Project 3 demonstrated that interpersonal differences in gut microbiota composition among defined bacterial collections from different healthy donors are coincidental with differences in conversions of GSPE, one of the BDPP components, to select biologically available phenolic acid metabolites. Of particular interest is the capability of bacteria from one of the donors to rapidly convert GSPE to DHCA, a BDPP-derived biologically available phenolic compound that Project 1 has found efficacious in promoting resilience to RSDS-mediated cognitive impairment. Outcomes from these initial studies support continued dissection of bacterial strains from Donor 1 to identify specific (or a subset of) bacteria that would facilitate conversion of GSPE to DHCA in gnotobiotic mouse models.

Research Cores: The execution of Research Projects 1-3 is supported by three Research Cores. The **Administrative-Biostatistics/Data Management Core A** provides administrative oversight to all components (Projects and Cores) of the BDSRC, as well as guidance on all issues related to study design and data entry, storage, analysis, and interpretation. The **Biosynthesis/Bioanalytical Core B** supports Research Projects 1- 3 by centralizing the procurement, synthesis, and quality assurance of dietary polyphenol botanical supplements, and by profiling the polyphenol compounds in target tissues. The **Behavioral Phenotyping and Electrophysiology Core C** provides behavioral phenotyping services for all the studies proposed in Research Projects 1-3. Moreover, it will assist and execute excitatory postsynaptic currents (EPSCs) and inhibitory postsynaptic currents (IPSCs) studies in Project 1 and long term potentiation (LTP) studies in Project 2.

Year 1 progress made by Cores A, B and C are as follows:

Core A: Core A has accomplished its primary goal to assess research progress and determine whether research goals are being met, while promoting transparent communication across Research Projects and Cores. In addition, Core A has facilitated the cross-project intellectual fertilization and synchronous collaborations while fostering new research among investigators of the ISMMS BDSRC, as well as among investigators from the two CANPITs and the two other BDSRCs.

Core B: The Biosynthesis Component of Core B provided standards and experiment samples to other components of the ISMMS BDSRC to support a wide variety of studies. In addition, the

Biosynthesis Component developed two approaches for the generation of glucuronidated dietary flavonoids: enzymatic biosynthesis and chemical synthesis. The Bioanalytical Component of Core B provided critical information on BDPP-derived phenols, their stability, and their bioavailability/metabolism in *in vitro* and *in vivo* studies. Moreover, the Bioanalytical Components has refined LC/UV/MS methods for improved quantitative analysis of BDPP polyphenols, including proanthocyanidins, anthocyanins, flavonols, and phenolic acids. Using these protocols, the Bioanalytical Component helped Project 3 analyzed *in vitro* conversions of GSPE by defined bacterial collections from different healthy donors to select biologically available phenolic acid metabolites and helped demonstrate interpersonal differences in the gut microbiota composition which significantly impacts conversion of GSPE to multiple biologically available BDPP-derived phenolic metabolites.

Core C: Core C has performed behavioral studies and electrophysiological analyses in support of Projects 1 and 2. These studies provided important functional assessments allowing Projects 1 and 2 to investigate the molecular and cellular mechanisms by which bioactive botanicals may beneficially promote psychological resilience in the context of social defeat (Project 1) and preserve cognitive function in the context of sleep deprivation (Project 2). Core C also provided toxicological assessments for select botanical compounds tested by Projects 1 and 2, which allow the potential benefits and safety of phenolic compounds to be determined for further preclinical and clinical translational applications.

C. Significance

The overall goals of the studies in this BDSRC are: (i) to mechanistically investigate the beneficial effect of the above botanical supplements in promoting resilience against psychological and physiological stress, (ii) to identify microbiota that are critical for the generation of bioactive phenolic metabolites related to resilience, and (iii) to develop next-generation probiotics capable of promoting bioavailability of the bioactive phenolic metabolites to enhance the efficacy of these botanical supplements in preserving psychological and cognitive resilience.

D. Future Plans

In Year 2, **Project 1** will continue with *in vitro* screening of additional BDPP-derived phenolic metabolites to identify individual (or a combination of) phenolic metabolites that are most efficacious and safe for simultaneous multi-targeting of inflammation and Rac 1 that ultimately will be tested *in vivo* in the RSDS mouse model in collaboration with Core C. Similarly, **Project 2** will continue with *in vitro* screening of additional BDPP-derived phenolic metabolites to identify individual (or a combination of) phenolic metabolites that are most efficacious and safe for simultaneous multi-targeting of CREB/mTOR signaling, LTP and/or IEG *in vitro*, and which will ultimately be tested *in vivo* in the SD mouse model in collaboration with Core C. Both **Projects 1 and 2** will continue to work with **Project 3** to provide bioactive phenolic compounds as potential targets for the development of next-generation probiotics for enhancing the efficacy of BDPP (and other polyphenol-rich botanicals) in preserving psychological and cognitive resilience. **Project 3** will use an in-house combinatorial screen technology to screen for and identify individual (or combinations of) human gastrointestinal microbial strains that are effective in metabolizing GSPE (or CGJ, or RSV) into bioactive phenolic metabolites of interest to Projects 1 and 2 for, respectively, the preservation of psychological and/or cognitive resilience. In addition, Project 3 plans to expand our *in vitro* metabolic potential screening experiments described above to additional healthy donor microbiotas to identifying a broader range of gut microbes capable of processing BDPP polyphenols into bioactive compounds. Moreover, Project 3 plans to initiate *in vivo* studies, in collaboration with Projects 1 and 2 and Core C, to test whether

inoculation of gnotobiotic mice with key microbial strains, identified *in vitro* to metabolize BDPP into bioactive, bioavailable compounds, will promote a) conversion of orally consumed BDPP to biologically relevant phenolic acids such as DHCA and b) accumulation of these phenolic acids in target tissues (e.g., blood and brain) following oral administration of BDPP.

Core A: In Year 2, Core A will continue organizing and keeping minutes of the regularly scheduled meetings among the ISMMS BDSRC Administrative Study Group, Internal Steering Committee the external advisory committees and the Study Coordination Work Groups. In addition, Core A will advertise and fund a new pilot research program based on merit of the science and relevance to the parent BDSRC goal. Core A will also teach MD/PhD students about the characterization of bioactive dietary polyphenols, with the ultimate goal of using this as a model for the evidence-based clinical development of naturally occurring products. Moreover, Core A will continue outreach by sharing findings through press releases for the public and by updating the Center website. Finally, the Core A Bioinformatics/Data Management component will implement more bioinformatic investigations to comprehensively cover three aspects: (1) to continue to employ the database and API for active data collection and management; (2) conducted metabolites selection based on *in vitro* readout (methods specified in grant application); (3) construct forest network (RFN) on multi-dimensional variables to determine causal mechanism of metabolite (R) intermediate phenotype (R) outcome.

Core B: The Core B Biosynthesis Component will continue to develop synthetic schemes for the full range of compounds needed, utilizing enzymatic and/or non-enzymatic approaches. The Core B Bioanalytical Component will continue providing support for individual Project needs with chemical profiling, quantification of metabolites bioavailability studies generated, quality control, and archiving of submitted active compounds/fractions. Using the GC/MS method developed in Year 1, the Bioanalytical component will continue to support each of the Research Projects in analyzing the profile and contents of a wide range of BDPP-derived phenolic metabolites among banked biological specimens, including bacterial broth and animal tissue specimens.

Core C: In Year 2, Core C will continue to provide state-of-the-art behavioral and electrophysiological assessments based on the goals of individual Projects with the overall objective of providing support to each Project that will allow the biological effects of bioactive botanicals on brain activity and function to be determined. Core C will continue to conduct toxicological assessments for all the individual botanical compounds used by each Project which will provide important information on the safety of individual botanicals to be readily translated to future preclinical and clinical applications.

E. Publications

Peer-refereed papers:

1. Menard CA, Pfau ML, Hodes GE, Russo SJ. Immune and Neuroendocrine Mechanisms of Stress Vulnerability and Resilience. *Neuropsychopharmacology*, 2016 (in press).
2. Golden SA, Christoffel DJ, Heshmati M, Guise K, Pfau ML, Aleyasin H, Hodes GE, Flanigan ME, Bregman D, Khibnik L, Tai J, Rebusi N, Krawitz B, Chaudhury D, Walsh JJ, Han MH, Shapiro ML, Russo SJ. Ventral striatal projections to the lateral habenula modulate aggression reward. *Nature*, 2016 (in press).
3. Wang J, Hodes G, Zhang HX, Zhang S, Kana V, Zhao W, Golden S, Bi WN, Menard C, Mazzola P, Xie M, Ho L, Han MH, Russo S, Pasinetti GM. Targeting inflammation and synaptic plasticity for the treatment of stress disorder and depression, 2016 (submitted).

4. Zhao W, Wang J, Bi W, Ferruzzi M, Yemul S, Freire D, Mazzola P, Ho L, Dubner L, Pasinetti GM. Novel application of brain-targeting polyphenol compounds in SD-induced cognitive dysfunction. *Neurochemistry International*. doi:10.1016/j.neuint.2015.07.023 (2015).
5. Ward L and Pasinetti GM. Recommendations for Development of Botanical Polyphenols as “Natural Drugs” for Promotion of Resilience Against Stress-Induced Depression and Cognitive Impairment. *NeuroMolecular Medicine*, 2016 (provisionally accepted).
6. T.S. Villani, E. Carry, H. Patel, G.M. Pasinetti, L. Ho, J. Faith, J.E. Simon and Q.L. Wu. 2016. Determination of the metabolism of grape derived products by intestinal microbiota using GC/MS (in preparation).
7. T.S. Villani, G.M.G. Ferruzzi, G.M. Pasinetti, J.E. Simon, Q.L. Wu. 2015. Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration. *Food Chem.* 170: 271-280 (cited as submitted in the original proposal, since then paper has been revised and published).

Abstracts of Presentations at Meeting:

1. **Wang J** and **Pasinetti GM**. Select polyphenol and/or phenolic acid metabolites promote psychological resilience to stress through attenuation of peripheral inflammatory responses. *Miami 2016 Winter Symposium: Inflammation*, Miami, FL, 2016.
2. **Wang J**. Novel interventions to promote resilience against age-associated psychological stress induced depression. Current Topics in Molecular Integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).
3. **Russo S**. Prophylactic interventions to promote resilience against stress-induced depression. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).
4. **Pasinetti GM**. Novel prophylactic and therapeutic interventions for stress-induced conditions, *Inaugural Reward Deficiency Syndrome Summit*, San Francisco, 2015.
5. **Pasinetti GM**. Role of Polyphenols in Promotion of Healthy Brain Aging, *Nutraceuticals in Neurodegeneration and Aging*, Singapore, 2015.
6. **Pasinetti GM**. From botanical sciences to neuroscience: how do we get there? *National Health Products Research Society Meeting 2016*, Charlottetown, Canada, 2016.
7. **Pasinetti GM**. Biomarkers associated with effect of polyphenols on SD-induced cognitive dysfunction. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).
8. **Pasinetti GM**. Optogenetic dissection of hippocampal mediated behavioral responses in the characterization of gene pathways associated with cognitive neuroresilience in response to SD in the aged. Current Topics in Molecular integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).
9. **Ho L** and **Pasinetti GM**. The role of intestinal microbiota in the characterization of natural drug pharmaceutical preparations in the treatment of major depressive disorder. *World Congress Expo on Pharmaceuticals and Drug Delivery Systems*, Dubai, 2016.
10. **Ho L**, **Faith J**, **Pasinetti GM**. Role of Intestinal Microbiota in the Preservation of Psychological and Cognitive Resilience. *Microbiome & Mice - Advancing Microbiome Research Symposium*, New York, NY 2016.
11. Llewellyn, SR, Britton GJ, Contijoch E, Mortha A, Merad M, **Faith JJ**. The effects of diet and the microbiota in determining host health. *Mucosal Immunology Course & Symposium*. Toronto, Canada 2016.

12. **Faith J.** Intestinal microbiota role in characterization of natural drugs in the treatment of major depression. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).
13. **Ho L.** Second generation microbiome probiotic drugs to promote resilience against age related dementia. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).
14. **Faith J.** Interactions among diet, botanicals, gut microbes, and healthy aging. Current Topics in Molecular integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).
15. **Ho L.** Role of intestinal microbiota in the promotion of psychological and cognitive resilience in neurodegenerative disorders. Current Topics in Molecular integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).

Symposia proposals for presentations at meetings:

1. **Pasinetti GM.** Symposium on “Current Topics in Molecular integrative Neuroresilience.” *International Society for Neurochemistry*, France, 2017 (submitted).
2. **Pasinetti GM.** Symposium on “Current Topics in Molecular integrative Neuroresilience.” *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).

F. Project-Generated Resources

Patents:

Provisional patent 62/286,649: Use of dihydrocaffeic acid and malvidin-3'-glucoside for the prevention and treatment of mood disorders. Wang J (2016)

PRESERVATION OF PSYCHOLOGICAL RESILIENCE UNDER CHRONIC STRESS – PROJECT 1, YEAR 1

A. Specific Aims

The Specific Aims of Project 1 have not changed from those originally proposed.

Aim 1: To investigate the effects of bioavailable phenolic metabolites derived from dietary polyphenol-rich botanical supplements on inflammatory signaling *in vitro* using circulating leukocytes and *in vivo* using a mouse model of RSDS.

Aim 2: To explore the central mechanisms through which bioavailable phenolic metabolites may beneficially influence RSDS induced synaptic restructuring and plasticity in the NAc.

Aim 3: To assess the impact of gut microbiome on the bioavailability of bioactive phenolic metabolites in promoting resilience in RSDS.

B. Studies and Results

For Aim 1 (year 1), we have screened 19 bioavailable phenolic metabolites derived from dietary polyphenol-rich botanical supplements on modulating inflammatory signaling using mouse peripheral blood mononuclear cells (PBMCs) stimulated with lipopolysaccharides. We identified three phenolic metabolites, 5'-O-methyl-epicatechin-3'-O-glucuronide, quercetin-glucuronide and 3,4-dihydrocaffeic acid (DHCA), which significantly reduced the LPS-induced IL-6 expression in PBMCs. Using the mouse model of RSDS *for in vivo* testing, we completed testing the role of DHCA in promoting resilience against chronic stress-induced depression in the mouse model of RSDS.

For Aim 2 (year 1), we have screened nine brain bioavailable phenolic metabolites for modulating synaptic mechanisms using primary medium spiny neuron (MSN)-enriched neuronal cultures. We identified one phenolic metabolite, malvidin-3'-glucoside (mal-gluc), that significantly increased the expression of Rac1 with a calculated half maximal effective concentration (EC50) of 3.52 nM. Rac1 expression in the NAc has been shown to be necessary and sufficient to promote social avoidance and anhedonia. We also tested the role of mal-gluc in promoting resilience against chronic stress-induced depression in the mouse model of RSDS.

For Aim 3 (year 1), DHCA has been selected as one of the target molecules whose bioavailability can be promoted through microbiome manipulation. In collaboration with Project 3, the identification of microbes important for the generation of DHCA is currently under active investigation.

C. Significance

In year 1, we have established the beneficial effect of oral supplementation with the bioactive dietary polyphenol preparation (BDPP) composed of grape seed polyphenol extract, Concord grape juice and resveratrol in promoting resilience in mouse model of stress disorder. We have identified select bioavailable and bioactive phenolic metabolites that can modulate peripheral inflammation and synaptic plasticity *in vitro*. We demonstrated that *in vivo* treatment with mal-gluc/DHCA can prophylactically promote resilience to RSDS-induced depression, and the benefits coincide with reduced peripheral IL-6 and normalization of synaptic plasticity, both structurally and functionally, in the NAc, a brain structure that plays a central role in the development of depression. Moreover, mal-gluc/DHCA is effective in treating depression phenotypes in RSDS-induced depression and in depression rendered by the transplantation of hematopoietic progenitor cells from stress-susceptible mice. Currently available antidepressant treatment is mainly based on the tricyclic antidepressants that target the serotonergic and/or noradrenergic system. Our studies provide the first experimental evidence that select botanical components such as mal-gluc and DHCA can promote resilience to stress-mediated psychological disorders in part, through targeting multiple key pathologic

mechanisms which are not addressed by classical antidepressants; therefore, our study provides a proof of concept and experimental evidence that botanical supplements can be used to target novel mechanisms to preserve and to promote psychological resilience under stressful conditions.

D. Future Plans

For the upcoming year 2, we will continue to screen the newly synthesized phenolic metabolites by Core B for anti-inflammatory activities and for modulating synaptic plasticity *in vitro*. We will continue the *in vivo* efficacy of positive phenolic metabolites identified using a mouse model of RSDS in collaboration with Core C for behavioral and electrophysiology assessments. We will continue to work with Project 3 to provide positive phenolic compounds that are potential targets for microbiome activity. All the proposed studies are in line with the proposal and there is no change in the program.

E. Publications

Peer-refereed papers:

8. Menard CA, Pfau ML, Hodes GE, Russo SJ. Immune and Neuroendocrine Mechanisms of Stress Vulnerability and Resilience. *Neuropsychopharmacology*, 2016 (in press).
9. Golden SA, Christoffel DJ, Heshmati M, Guise K, Pfau ML, Aleyasin H, Hodes GE, Flanigan ME, Bregman D, Khibnik L, Tai J, Rebusi N, Krawitz B, Chaudhury D, Walsh JJ, Han MH, Shapiro ML, Russo SJ. Ventral striatal projections to the lateral habenula modulate aggression reward. *Nature*, 2016 (in press).
10. Wang J, Hodes G, Zhang HX, Zhang S, Kana V, Zhao W, Golden S, Bi WN, Menard C, Mazzola P, Xie M, Ho L, Han MH, Russo S, Pasinetti GM. Targeting inflammation and synaptic plasticity for the treatment of stress disorder and depression, 2016 (submitted).

Abstracts for presentations at meetings:

1. **Wang J** and **Pasinetti GM**. Select polyphenol and/or phenolic acid metabolites promote psychological resilience to stress through attenuation of peripheral inflammatory responses. *Miami 2016 Winter Symposium: Inflammation*, Miami, FL, 2016.
2. **Wang J**. Novel interventions to promote resilience against age-associated psychological stress induced depression. Current Topics in Molecular integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).
3. **Russo S**. Prophylactic interventions to promote resilience against stress-induced depression. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).

F. Project-Generated Resources

Patents:

Provisional patent 62/286,649: Use of dihydrocaffeic acid and malvidin-3'-glucoside for the prevention and treatment of mood disorders. Wang J (2016)

PROMOTION OF RESILIENCE AGAINST COGNITIVE DECLINE INDUCED BY SLEEP DEPRIVATION – PROJECT 2, YEAR 1

A. Specific Aims

The Specific Aims of Project 2 have not changed from those originally proposed.

Aim 1: To continue to explore the mechanistic role of brain-bioavailable phenolic metabolites on signal transduction pathways associated with promotion of cognitive resilience in a mouse model of SD.

Aim 2: To explore mechanistically the role of brain-bioavailable phenolic metabolites in promoting cognitive resilience through modulation of IEG expression *in vitro* and *in vivo using novel optogenetics technology*.

Aim 3: To explore the role of novel next-generation probiotics designed to promote conversion of select bioactive phenolic metabolites from botanical supplement mixture to enhance bioavailability and bioactivity and promote cognitive resilience in response to SD.

B. Studies and Results

For Aim 1 (Year 1), we have screened six brain-bioavailable phenolic metabolites derived from dietary polyphenol-rich botanical supplements in promoting CREB signaling pathways using primary cortico-hippocampal neurons. We have identified two phenolic metabolites, Quercetin-glucuronide (Quer-Gluc) and Malvidin-glucoside (Mal-Gluc), that can promote CREB phosphorylation through activation of CamKII and mTOR signaling pathways. We tested the *in vivo* efficacy of Quer-Gluc and Mal-Gluc in promoting cognitive function in a mouse model of acute SD.

For Aim 2 (Year 1), we have screened 11 bioavailable phenolic metabolites for modulating immediate early gene (IEG) expression using primary cortico-hippocampal neurons following brain derived neurotrophic factor (BDNF) stimulation. We identified four phenolic metabolites, delphinidin-glucoside, Quer-gluc, Mal-gluc and homovanillic acid (HVA), that can promote the expression of C-fos and Arc which will be tested *in vivo* to investigate memory engrams following SD using optogenetic technology. We have also established the c-fos-tTA transgenic mice colony which will be injected with TRE-ChR2-mCherry virus in the hippocampal formation to test the effect of SD on cognitive impairments and the beneficial role of select phenolic metabolites capable of promoting IEGs in promoting resilience to sleep-deprivation mediated cognitive dysfunction.

For Aim 3 (Year 1), HVA can be one of the target molecules whose bioavailability can be promoted through microbiome manipulation. In collaboration with Project 3, the identification of microbes important for the generation of HVA will be actively investigated.

C. Significance

In Year 1, we have identified select bioavailable and bioactive phenolic metabolites that can promote resilience to cognitive impairments under the context of SD stress. We demonstrated that *in vivo* treatment with Quer-Gluc precursor quercetin and Mal-Gluc can promote cognitive function following acute SD in part through the activation of two independent yet interrelated pathways: CREB signaling pathway and mTOR signaling pathway. Our studies provide the experimental evidence that quercetin and Mal-Gluc can selectively modulate signaling pathways that are interrupted by SD. Given the safety and tolerability of these two botanical compounds, our preclinical study has provided a basis for potential translational application of select botanicals in promoting resilience to the SD-induced cognitive deficits targeting the CREB signaling and mTOR signaling pathways.

D. Future Plans

For the upcoming Year 2, we will continue to screen the newly synthesized phenolic metabolites by Core B for promoting CREB signaling and IEGs *in vitro*. We will continue to test the *in vivo* efficacy of positive phenolic metabolites identified using a mouse model of SD in collaboration with Core C for behavioral and electrophysiology assessments. We will test the role of select bioavailable and bioactive phenolic metabolites in promoting cognition using optogenetic technology. We will continue to work with Project 3 to provide positive phenolic compounds for the identification of microbial strains that are capable of generating these phenolic compounds. All the proposed studies are in line with the proposal and there is no change in the program.

E. Publications

Peer-refereed papers:

1. Zhao W, Wang J, Bi W, Ferruzzi M, Yemul S, Freire D, Mazzola P, Ho L, Dubner L, Pasinetti GM. Novel application of brain-targeting polyphenol compounds in SD-induced cognitive dysfunction. *Neurochemistry International*. doi:10.1016/j.neuint.2015.07.023 (2015).

Abstracts of Presentations at Meeting:

1. **Pasinetti** GM. Novel prophylactic and therapeutic interventions for stress-induced conditions, *Inaugural Reward Deficiency Syndrome Summit*, San Francisco, 2015.
2. **Pasinetti** GM. Role of Polyphenols in Promotion of Healthy Brain Aging, *Nutraceuticals in Neurodegeneration and Aging*, Singapore, 2015.
3. **Pasinetti** GM. From botanical sciences to neuroscience: how do we get there? *National Health Products Research Society Meeting 2016*, Charlottetown, Canada, 2016.
4. **Pasinetti** GM. Biomarkers associated with effect of polyphenols on SD-induced cognitive dysfunction. Current Topics in Molecular integrative Neuroresilience Symposium. International Society for Neurochemistry, France, 2017 (submitted).
5. **Pasinetti** GM. Optogenetic dissection of hippocampal mediated behavioral responses in the characterization of gene pathways associated with cognitive neuroresilience in response to SD in the aged. Current Topics in Molecular integrative Neuroresilience. International College of Geriatric Psychoneuropharmacology, Greece 2017 (submitted).

F. Project-Generated Resources

N/A

ROLE OF THE MICROBIOME IN PROMOTION OF COGNITIVE AND PSYCHOLOGICAL RESILIENCE – PROJECT 3, YEAR 1

A. Specific Aims

The Specific Aims of Project 3 have not changed from those originally proposed.

Aim 1: To explore the delivery and accumulation of phenolic metabolites from the polyphenol-rich bioactive mixture of dietary botanical supplements in the context of human microbiota. Aim 2: To identify bacterial strains from human microbiota that are necessary and sufficient for the processing and bioavailability of phenolic acids from the botanical mixture.

Aim 3: To explore the use of a next-generation probiotic cocktail to increase the bioavailability of phenolic acids from the botanical mixture.

B. Studies and Results

Although originally planned for years 2-3 (Aim 2), we have made substantial progress generating arrayed culture collections of gut bacteria from diverse humans. These arrayed culture collections consist of banked 96-well glycerol stocks of bacteria, where each well in the plate contains a unique bacterial strain isolated from a single human, providing the opportunity to identify the functional capacities of individual microbes and collections of microbes across individuals. Our original methods for generating these culture libraries required identifying each bacterial species using next-generation sequencing methods. By switching this identification to a MALDI-TOF mass spec based approach, we can now identify microbial species in real-time. This advance has enabled us to move from isolating human microbiota culture collections from a human microbiota over the course of 1-3 months down to 1-2 weeks. As such, we now have ~1000 unique strains of bacteria isolated from different humans. The availability of these unique resources a year in advance allowed us to accelerate our experimental plans. We had originally planned to screen human donor feces from different individuals to determine the variability in polyphenol bioprocessing by the microbiome across individuals. However, **our ultimate goal is the identification of culturable gut microbes that could form a next-generation probiotic cocktail to produce bioactive polyphenols and phenolic acids *in vivo*. These culture collections allow us to skip the initial experiments in feces and proceed directly towards our goal of identifying this next-generation probiotic of culturable organisms.** The earlier we can identify such organisms the more time Projects 1-3 will have to explore the functional capacity of such microbes to enable the potential of grape based dietary botanical preparations to provide beneficial effects to the host. To capitalize on these unique resources, we have begun some exciting *in vitro* experiments to identify the metabolic potential of culturable microbial communities isolated from different humans to produce bioactive polyphenols and phenolic acids through the metabolism of BDPP components – grape seed polyphenol extract (GSPE), Concord grape juice (CGJ) and resveratrol (RSV).

***In vitro* metabolic processing of polyphenols from GSPE, CGJ, and RSV by culturable human microbiota collections**

Overall Study Design: Defined culture collections of ~20 bacteria strain isolates from three different human donors (~60 total bacterial strains) are being assayed *in vitro* for their capacity to metabolize GSPE, GGJ, and RSV to biologically available phenolic acid compounds. The current ongoing experiments inoculate culture media with a rich bacterial culture collection from individual donors, in the presence / absence of a total polyphenol extract from GSPE, CGJ, or RSV. Conditioned broth are

recovered at 24 h and at 72 h and are then sent to Dr. James Simon, Rutgers University (Core B Analytical Component) for analysis.

C. Significance

We have completed an *in vitro* study investigating interpersonal differences in gut microbiota composition in converting GSPE to biologically available phenolic acids. We have previously demonstrated that polyphenol components from GSPE can pass intact through the upper gastrointestinal track and are available in the colon for gut microbial metabolism (1). Thus, we compared defined bacterial collections from different healthy donors for their capability of converting GSPE to phenolic acids, *in vitro*. Bacteria broth (5-ml) was inoculated with defined bacterial collections from three healthy donors. GSPE (600 mg/L) was then added. Contents of GSPE polyphenols and GSPE-derived phenolic acid metabolites in the bacterial broth were assessed at 24 or 72 h. Results from our *in vitro* study confirming interpersonal differences in bacterial strains from the three donors are coincidental with differences in conversions of GSPE to select biologically available phenolic acid metabolites. Of particular interest is the capability of bacteria from Donor 1 to rapidly convert GSPE to dihydrocaffeic acid (DHCA). Recent evidence from Project 1 has implicated the potential contribution of DHCA in promoting psychological resilience in a mouse model of repeated social defeat stress. Outcomes from these initial studies support continued dissection of bacterial strains from Donor 1 to identify specific (or a subset of) bacteria that would facilitate conversion of GSPE to DHCA in gnotobiotic mouse models.

D. Future Plans

Combinatorial screen to identify microbial strains that metabolize GSPE into bioactive compounds. By the end of Year 2, we aim to identify the particular microbial strains or groups of strains in Donor 1 that are capable of metabolizing, *in vitro*, GSPE to DHCA (as well as metabolizing GSPE into additional bioactive, biologically available metabolites of interest for Projects 1 and 2). We will use *in vitro* combinatorial bacterial and statistical modeling to dissect the interrelationship between the gut microbiota and conversion of biologically relevant phenolic acid GSPE. In addition, we aim to initiate studies to identify if the metabolic conversion of GSPE to DHCA is replicated in preclinical gnotobiotic mouse studies using animals with and without the identified strains capable of this conversion. By the end of Year 2, we also aim to identify human microbial strains that are capable of converting CGJ and RSV to phenolic metabolites of interest for Projects 1 and 2.

Based on our previous work identifying key effector microbes from culture collections of gut bacteria (2), we anticipate our high throughput screening studies will find bacteria in the culture community that individually or in combination can generate key metabolites in a screen of roughly 100 culture combinations per donor library. To do so, we will use our anaerobic liquid handling robot to randomly fractionate the bacteria in the community into subsets of varying sizes (e.g., 2 strains, 3 strains, etc.). For example, to identify bacteria strains from Donor 1 that are capable of converting GSPE to DHCA, Donor 1 bacterial community will be provided the same culture media spiked with GSPE as in our complete human microbiota culture experiments detailed above. As described in the P50 grant proposal, we will then use computational models to identify the organisms whose presence or absence in the culture best explains the existence of the metabolites of interest. Finally, we will take the key microbes, re-culture them, and validate them individually or with the appropriate community members cultured with the GSPE. The identified microbial effector strains or groups of strains capable of metabolizing GSPE to DHCA *in vitro* will serve as the initial microbial therapeutic test community provided to gnotobiotic animals to validate *in vivo* the essential role of specific microbial taxa on the generation of bioactive GSPE derived metabolites.

Expanding BDPP bioprospecting to additional human microbiotas. We also plan to lay the ground work to expand our *in vitro* metabolic potential screening experiments described above to four additional healthy donor microbiotas to identifying a broader range of gut microbes capable of processing BDPP polyphenols into bioactive compounds. Towards this, we are currently generating new culture collections from four additional healthy individuals whose microbes were previously used in fecal microbiota transplant therapies in humans.

Optimizing dosing and metabolic assays in vivo. Our larger goal is to test whether inoculation of gnotobiotic mice with key microbial strains, identified *in vitro* to metabolize BDPP into bioactive, bioavailable compounds, will promote a) conversion of orally consumed BDPP to biologically relevant phenolic acids such as DHCA and b) accumulation of these phenolic acids in target tissues (e.g., blood and brain) following oral administration of BDPP. In preparation of these gnotobiotic mice studies, we will be treating wild-type mice with BDPP (200 mg/kg bw/day GSPE, 57 mg/kg bw/day total polyphenol extract from CGJ, and 300 mg/kg bw/day RSV), as we have done in the past (3, 4).

Mice will be treated for 10 days to simulate chronic administration. Biological specimens (e.g., feces, urine, colon, blood, brain) will be collected at the end of the study. These specimens will be forward to Dr. James Simon (Rutgers University, Core B Analytical component), for Core B to optimize its tissue extraction and analytical protocol for quantitative analysis of phenolic acids from BDPP. Outcomes from these studies will allow for the optimal design of the *in vivo* studies involving gnotobiotic mice. To optimize the use of BDPP in gnotobiotic and germ-free conditions, we will determine the baseline absorption of BDPP in germ-free mice devoid of secondary metabolism by the gut microbiota. These experiments will be performed using the same design as the conventional mouse experiment, except the animals will be germ-free and housed in a flexible film gnotobiotic isolator.

Collectively, outcomes from our Year 2 studies will provide the basis for developing next-generation probiotic cocktails to increase the bioavailability of bioactive phenolic metabolites from BDPP. In Year 3 in collaboration with Projects 1 and 2, we will then test whether introduction of these probiotic cocktails in mice will boost the beneficial effects of BDPP in promoting cognitive and/or psychological resilience.

E. Publications

Peer-refereed papers:

None

Abstracts for Presentations at Meetings:

1. **Ho L** and **Pasinetti GM**. The role of intestinal microbiota in the characterization of natural drug pharmaceutical preparations in the treatment of major depressive disorder. *World Congress Expo on Pharmaceuticals and Drug Delivery Systems*, Dubai, 2016.
2. **Ho L**, **Faith J**, **Pasinetti GM**. Role of Intestinal Microbiota in the Preservation of Psychological and Cognitive Resilience. *Microbiome & Mice - Advancing Microbiome Research Symposium*, New York, NY 2016.
3. Llewellyn, SR, Britton GJ, Contijoch E, Mortha A, Merad M, **Faith JJ**. The effects of diet and the microbiota in determining host health. Mucosal Immunology Course & Symposium. Toronto, Canada 2016.
4. **Faith J**. Intestinal microbiota role in characterization of natural drugs in the treatment of major depression. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).

5. **Ho L.** Second generation microbiome probiotic drugs to promote resilience against age related dementia. Current Topics in Molecular integrative Neuroresilience Symposium. *International Society for Neurochemistry*, France, 2017 (submitted).
6. **Faith J.** Interactions among diet, botanicals, gut microbes, and healthy aging. Current Topics in Molecular integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).
7. **Ho L.** Role of intestinal microbiota in the promotion of psychological and cognitive resilience in neurodegenerative disorders. Current Topics in Molecular integrative Neuroresilience. *International College of Geriatric Psychoneuropharmacology*, Greece 2017 (submitted).

F. Project-Generated Resources

N/A

References

1. D. Wang *et al.*, *Mol. Nutr. Food Res.* **59**, 1025 (2015).
2. J. J. Faith, P. P. Ahern, V. K. Ridaura, J. Cheng, J. I. Gordon, *Sci. Transl. Med.* **6**, 220ra11 (2014).
3. W. Zhao *et al.*, *Neurochem. Int.* (2015).
4. J. Wang *et al.*, *Front Aging Neurosci.* **6**, 42 (2014).

ADMINISTRATIVE-BIOSTATISTICS/DATA MANAGEMENT CORE – CORE A, YEAR 1

A. Specific Aims

The Specific Aims of the Administrative - Biostatistics/Data Management Core A have not changed from those originally proposed.

Aim 1: By continually monitoring and evaluating the scientific progress of the BDSRC and enhancing collaboration among its Research Projects and Cores.

Aim 2: By providing oversight through academic leadership and coordination in the administration of fiscal and personnel issues of the BDSRC among the participating institutions and their investigators.

Aim 3: By fostering new research, developing young investigators, and bringing new vision to the challenges in the development of botanical supplements for brain wellness and mental health

Aim 4: By providing biostatistical support, data storage, and scientific communication

B. Studies and Results

In Year 1, no changes to the aims or budget were made in this Core A awarding component.

Aim 1: In Year 1, Core A organized and kept minutes of the monthly Administrative Study Group meetings, whose main goal is to assess the Research Projects' progress and determine whether research goals are being met. In addition, the Administrative Work Group ensures appropriate budgetary spending and monitors other fiscal matters related to the research operations by Research Projects and Cores, ultimately ensuring compliance with the proposed spending plan, including subcontracts.

Internal Steering Committee

Core A organized and kept minutes of the monthly Internal Steering Committee meetings whose main goal is to promote integration among the Research Projects and Cores and to resolve any obstacles to the fulfillment of the projected goals. The meetings were held either in person, through teleconferences, or through video conference.

External Advisory Board (EAC)

Core A established the EAC to monitor the progress of the projects, to advise the Internal Steering Committee, and to participate in the strategy planning of the ISMMS BRC. The EAC is chaired by Dr. Ming Hu, Professor of Pharmaceutics at the University of Houston, and also includes Dr. Tracy Bale, Associate Professor of Neuroscience at the Perelman School of Medicine, Dr. Ronald Duman, Professor of Psychiatry and Yale School of Medicine, Dr. David Dinges, Chief of Sleep and Chronobiology at the Perelman School of Medicine, and Dr. Federico Re, and Assistant Professor of Bacteriology at the University of Wisconsin – Madison. These individuals adequately cover the diverse applications of this BRC from the bioavailability of drugs to characterization of the molecular actions of stress to the neurophysiology of circadian and sleep biology to the role of microbial communities. The EAC had its first teleconference to discuss the Projects and introduce the overall goal of the ISMMS BRC activities met as per RFA recommendations within the three months of the start of the budget period. The EAC members will attend a symposium organized at ISMMS on June 15th with the primary goal to review the scientific activities accomplished in Year 1 by the ISMMS BRC, during which all the investigators of the ISMMS BRC will present their scientific activities of Year 1. The goal of this symposium is to have the EAC review and provide guidance on Center activities. We expect the EAC to comment and eventually to report on Center operations in Year 1 in particular in relation to the appropriate allocation of resources devoted to the most scientifically

worthy and significant projects, and to ensure that maximal synergy was achieved. The EAC will provide minutes to NIH staff within 30 days of the symposium on the performance of the ISMMS BRC Year 1 activities.

Aim 2: In Year 1, Core A organized the meetings and took minutes of the Study Coordination Work Group meetings, whose main goal is to allow the ISMMS BRC investigators to fully and rapidly share ideas, to address questions related to experimental work, and to synchronize efforts. This work group meets monthly to discuss recent findings and also to facilitate the ongoing exchange of information regarding mechanisms and bioactivities related to the phenolic botanical supplements being investigated in the early phases of the studies. There is representative leadership from all the Research Projects and Cores at each meeting.

Aim 3: Pilot Research Program. In Year 1, Core A largely advertised the pilot program funding opportunity throughout Mount Sinai, collaborating institutions, as well as other institutions in the CARBON. Core A received eight applications and after a first administrative review only seven met compliance for further review. The seven applications were reviewed by leaders and co-leaders of the ISMMS BRC, who were instructed that the primary consideration for evaluation was based on merit of the science and relevance to the parent BRC goal. Based on these review criteria, four applications were streamlined and the three applications with the highest impact score were shared with the external advisory committee, which re-reviewed the applications and provided further recommendations. The three applications were then reviewed by the CARBON Director who agreed that Dr. Stephen Salton's application entitled "Antidepressant and pro-cognitive efficacy of cocoa- and grape-derived polyphenols is dependent on hippocampal BDNF/TrkB signaling" should receive pilot program funding for one year.

Course on Molecular Integrative Neuroresilience. Dr. Pasinetti (PD/PI) and Dr. Ho (Project 3 Leader) co-directed a course for eight MD/PhD students at the Icahn School of Medicine at Mount Sinai entitled "Current Topics in Molecular integrative Neuroresilience." The course focused on understanding the mechanisms by which specific dietary polyphenols may protect against psychological stress, while also clarifying interactions between gastrointestinal microbiome and dietary polyphenols in the promotion of neuroresilience. Collectively, this course explored and discussed the characterization of bioactive dietary polyphenols, with the ultimate goal of using this as a model for the evidence-based clinical development of naturally occurring products. The course involved guest lectures from ISMMS Center Core and Project leaders including Dr. Russo (Project 1 Leader), Dr. Faith (Project 3 Leader), and Dr. Dixon (Core B Leader).

Supplement to promote diversity in health-related research. Dr. Pasinetti submitted a supplement to the NCCIH to train Mr. Justin Brathwaite, a graduate of Columbia University, to intellectually contribute to the investigation of molecular mechanisms that may underlie the promotion of resilience to sleep deprivation (SD)-mediated cognitive impairment through treatment with certain polyphenol botanicals. Mr. Brathwaite will contribute in the investigation of brain bioavailable bioactive polyphenol metabolites which will be discovered during the screening process of the botanical supplement mixture. Ultimately, Mr. Brathwaite will be trained in optogenetics technology which will allow the visualization of potential novel molecular mechanisms consistent with the theme of Project 2 of the BRC, without duplicating the current proposal, but rather extending the research on how polyphenol botanicals may promote resilience to SD-mediated cognitive impairment. Collectively, Mr. Brathwaite, through this training, will reach the necessary mass of technical information as well as intellectual

involvement that will enable him to continue independently in the understanding of novel identified phenolic metabolites.

Outreach Work Group. Core A established the ISMMS BRC website to provide supportive outreach to both the scientific community as well as the public in order to increase awareness of the BRC's research efforts and the implications of our findings in the field of botanical supplements and the promotion of cognitive and psychological resiliency. Core A has also published two press releases summarizing recent evidence from the ISMMS BRC using lay language to disseminate the research findings to the interested public. In addition, Dr. Pasinetti has submitted two symposiums, one to the International Society for Neurochemistry meeting and one to the International College of Geriatric Psychoneuropharmacology meeting, on Current Topics in Molecular integrative Neuroresilience. These symposiums are designed to discuss strategies to safely promote resilience against stress-induced cognitive and psychological impairment at the mechanistic level.

Aim 4: In Year 1, Core A developed databases (MySQL) and database tables to host data generated by Projects and Cores. The databases are able to host multiple data types (quantitative, categorical, etc), and allow efficient search and downstream analysis. API (application programming interface) was also developed for authorized users to upload and query data. Further, Core A conducted extensive validation on the integrity of the tables and API.

Collaboration with UIC CANPIT (PI: Guido Pauli): The principal objective of the collaboration between the UIC CANPIT and the ISMMS BRC is the production of designer knock-out (KO) extracts for Concord Grape Juice (CGJ) to be depleted of malvidin glucoside. The second set of objectives for the collaboration is associated with comparative NMR fingerprinting of botanicals, including eventually metabolomics analysis, quantification, and purity determination of major bioactive compounds. In Year 1, purity determination through qHNMR analysis together with ¹H NMR fingerprinting has been performed for a synthetic resveratrol sample and a grape seed extract, respectively. This work is still in progress for optimization.

Other Collaborations: Core A established collaborations with the other CANPITs and Botanical Centers. The four active collaborations include:

1. Pennington/Rutgers BRC (PI: Will Cefalu) – The principal objective of the collaboration between the Pennington/Rutgers BRC and the ISMMS BRC is to measure parameters in brains in response to certain dietary botanical treatments related to glycemic control and to explore the molecular pathways in stress response and cognitive function.

2. UIC BRC (PI: Richard Van Breemen) – The principal objective of the collaboration between the UIC BRC and the ISMMS BRC is to take isolated bacteria and spike mixture with dietary products to see if we can generate active ingredients.

3. University of Texas Southwestern CANPIT (PI: MacMillan) – The primary objective of the collaboration between the UTSW CANPIT and the ISMMS BRCS is to predict the bioactivities and mechanisms of actions of individual polyphenol metabolites using cytological profiling analysis and Functional Signature of Ontology. In Year 1, UTSW CANPIT has received a series of compounds from the ISMMS BRC and screened them in cytological profiling.

C. Significance

In Year 1, Core A has accomplished its primary goal to assess research progress and determine whether research goals are being met, while promoting transparent communication across Research Projects and Cores. In addition, Core A has facilitated the cross-project intellectual fertilization and synchronous collaborations while fostering new research among investigators of the ISMMS BRC, as well as among investigators from the two CANPITs and the two other BRCs.

D. Future Plans

In Year 2 with respect to Aim 1, Core A will continue organizing and keeping minutes of the monthly 1) Administrative Study Group meetings to assess the Research Projects' progress and determine whether research goals are being met, 2) Internal Steering Committee meetings to promote integration among the Research Projects and Cores and to resolve any obstacles to the fulfillment of the projected goals, and 3) EAC meetings to monitor the progress of the projects, to advise the Internal Steering Committee, and to participate in the strategy planning of the ISMMS BRC. With respect to Aim 2, Core A will continue organizing and keeping minutes of the Study Coordination Work Group meetings to ensure ISMMS BRC investigators to fully and rapidly share ideas, to address questions related to experimental work, and to synchronize efforts. With respect to Aim 3, Core A will advertise and fund a new pilot research program based on merit of the science and relevance to the parent BRC goal with input from ISMMS BRC investigators, the EAC, and the Program Director. Core A will also teach MD/PhD students about the characterization of bioactive dietary polyphenols, with the ultimate goal of using this as a model for the evidence-based clinical development of naturally occurring products. Finally, Core A will continue outreach by sharing findings through press releases for the public and by updating the Center website. With respect to Aim 4, Core A Bioinformatics/Data Management will implement more bioinformatic investigations. To achieve this goal, we added a new personnel to Core A specialized in bioinformatics who will use the random forest network (RFN) as the framework to determine the inter-relationship among the multi-dimensional variables planned to be assessed by Projects and Cores, including the identified bioactive metabolites, amount, concentration, purity of the metabolites, molecular phenotype (e.g. high-throughput expression profiles), and endpoints. Random forests is a notion of the general technique of random decision forests that are an ensemble learning method for classification, regression and other tasks, that operate by constructing a multitude of decision trees. The edges identified by RNF also will allow us to identify direction of regulation therefore allow causal inference. Therefore, in Year 2 Core A will focus on three aspects (1) to continue to employ the database and API for active data collection and management; (2) conducted metabolites selection based on in vitro readout (methods specified in grant application); (3) construct RFN on multi-dimensional variables to determine causal mechanism of metabolite (R) intermediate phenotype (R) outcome.

E. Publications

Peer-refereed papers:

1. Ward L and Pasinetti GM. Recommendations for Development of Botanical Polyphenols as "Natural Drugs" for Promotion of Resilience Against Stress-Induced Depression and Cognitive Impairment. *NeuroMolecular Medicine* (provisionally accepted, 2016).

Symposia proposals for presentations at meetings:

1. Pasinetti GM. Symposium on "Current Topics in Molecular integrative Neuroresilience." *International Society for Neurochemistry*, France, 2017 (submitted).

2. Pasinetti GM. Symposium on “Current Topics in Molecular integrative Neuroresilience.”
International College of Geriatric Psychoneuropharmacology, Greece 2017 (submitted).

F. Project-Generated Resources

N/A

BIOSYNTHESIS/BIOANALYTICAL CORE – CORE B, YEAR 1

A. Specific Aims

The Specific Aims of Core B have not changed from those originally proposed.

Project 1: Preservation of psychological resilience under chronic stress

Project 2: Promotion of resilience against cognitive decline induced by sleep deprivation

Project 3: Role of the microbiome in promotion of cognitive and psychological resilience

B. Studies and Results

B.1 Biosynthesis Component: Compounds to be synthesized fall into four classes of phenolic compounds: flavanol, flavonol, anthocyanin and stilbene. Those glucuronidated and methylated derivatives of these compounds found in plasma and brain of animals fed GSPE not commercially available will be synthesized by Core B. In Y1, the compound 500 mg of dihydrocoumaric acid, from microbial degradation of dietary anthocyanins, was synthesized by catalytic reduction. We have developed two approaches for the generation of glucuronidated dietary flavonoids: enzymatic biosynthesis and chemical synthesis. For enzymatic synthesis, we have previously shown the utility of recombinant human glucuronosyl transferase UGT1A9 for the synthesis of glucuronidated forms of catechin, epicatechin and their 3'-O-methyl derivatives. We obtained and tested 9 recombinant human UGTs against epicatechin and quercetin; UGT1A9 remains the most efficient enzyme for production of epicatechin glucuronides, whereas UGT1A1 is also active with quercetin. Studies to evaluate the remaining enzymes are in progress. We are also developing approaches for optimizing the yields of the enzymatic reactions.

B.2 Bioanalytical Component: *Quality Control of Grape Derived Products:* GSPE, CGJ, and RSV, contain a complex mixture of proanthocyanidins, anthocyanins and other polyphenols including flavonols, resveratrol, and phenolic acids. LC/UV/MS methods developed were used to quantitate the array of grape polyphenols including proanthocyanidins, anthocyanins, flavonols, and phenolic acids in GSPEs, epicatechin enriched GSPE, original and concentrate CGJs and the grape juice placebo for projects 1, 2 and 3. Total polyphenols of the all grape-derived samples were assayed using UV-Vis spectrometry. Results allowed for the refinement of target compounds for further investigation of bioavailability and bioactivity. Available reference compounds (19), as well as the synthetic dihydrocoumaric acid were analyzed for purity using HPLC/UV/MS or ¹H- and ¹³C-NMR (for extremely high polar compounds of Mandelic Acid and L-(+)-tartaric acid). The single compounds were all highly pure and acceptable for use by the research projects. Stability of the individual compounds 3-(3,4-dihydroxyphenyl)propionic acid (DHPA); malvidin-3-O-glucoside (oenin) and quercetin were evaluated. Results indicated that no significant degradation was observed over a 5-day period (6 technical reports delivered).

Determination of the Metabolism of Grape Derived Polyphenols by Intestinal Microbiota: Samples from fermentation studies at Mt. Sinai using GSPE and CGJ and 3 bacterial donors were evaluated using a GC/MS method developed at Rutgers to quantitate content of bacterial metabolites. For the GSPE treated samples, the bacterial broth samples showed consistent results between same-donor repetitions, and results demonstrated a time-based effect on metabolite profile. There were clear differences between the metabolic activities of the three bacterial donor groups. Broths from donors 1 and 2 showed near complete consumption of gallic acid even after 24h; whereas broth from donor 3 did not consume a significant quantity of gallic acid, even after 72h. Spiked recovery experiments demonstrated the reproducibility and accuracy of this method. For CGJ treated samples, bacterial broth samples also showed consistent results between same-donor repetitions. Results demonstrated a time-based effect on metabolite profile. There were clear differences between the metabolic activity

of the three bacterial donor groups. Broths from donor 3 showed increasing content of caffeic acid over 72h, whereas broth from donor 1 and 2 showed a consumption of a significant quantity of caffeic acid, after 72h (2 technical reports delivered).

C. Significance

C.1 Biosynthesis Component: Phase II metabolites of dietary polyphenols by the **Biosynthesis Component** will provide standards and experimental samples with application in a wide variety of studies.

C.2 Bioanalytical Component: Data from **Bioanalytical Component** provided critical information on grape derived phenols, their stability, and their bioavailability/metabolism in *in vitro* and *in vivo* studies.

D. Future Plans

D.1 Biosynthesis Component: We developed synthetic schemes for the full range of compounds needed. Most are multi-step, in which the base flavonoid structure is assembled de novo, and involve addition and removal of protecting groups. The advantage of the synthetic schemes over the enzymatic approach is that it is possible to synthesize non-biological isomers.

D.2 Bioanalytical Component: We will continue providing support for individual projects needs with chemical profiling, quantification of metabolites bioavailability studies generated, QC, and archiving of submitted active compounds/fractions. Using the GC/MS method developed in Y1, we will support for Project 3 in quantifying the content of bacterial metabolites achieved by different bacterial donors and different grape derived materials including GSPE, CGJ and resveratrol. Samples will be also examined as needed for their stability over time to predict qualitative changes stored under different conditions. Core B is currently conducting method development for bioanalysis of phase II metabolites such as glucuronide conjugates of (3'-Me-) catechin and (3'-Me-) epicatechin. This method will be utilized to determine the metabolites in the plasma and brain samples obtained from animals treated with epicatechin-enriched extract for Project 2. In further support of Projects 1 and 2, methods to assess bioavailability and metabolism of other biological samples treated with GJ and resveratrol will be conducted. Combined with GC/MS bioanalysis on the Phase I metabolites, these approaches will expand our knowledge on qualitative and quantitative grape polyphenol profiles in plasma and tissue distribution. Additionally, **Bioanalytical component** will distribute an initial LIMS system to the Center for testing. as an online tracking system.

E. Publications

Peer-refereed papers:

1. T.S. Villani, E. Carry, H. Patel, G.M. Pasinetti, L. Ho, J. Faith, J.E. Simon and Q.L. Wu. 2016. Determination of the metabolism of grape derived products by intestinal microbiota using GC/MS (in preparation).
2. T.S. Villani, G.M.G. Ferruzzi, G.M. Pasinetti, J.E. Simon, Q.L. Wu. 2015. Chemical investigation of commercial grape seed derived products to assess quality and detect adulteration. Food Chem. 170: 271-280 (cited as submitted in the original proposal, since then paper has been revised and published).

Abstract for Presentations at Meetings:

1. Thomas Villani, Eileen Carry, Harna Patel, Giulio M. Pasinetti, Lap Ho, Jeremiah Faith, James Simon and Qingli Wu. 2016. Investigation into the effects of intestinal microbiota on the

metabolism and uptake of grape derived products using GC/MS. 252 Annual Meeting of American Chemical Society. August 21-25, Philadelphia, PA.

F. Project-Generated Resources

N/A

BEHAVIORAL PHENOTYPING AND ELECTROPHYSIOLOGY CORE – CORE C, YEAR 1

A. Specific Aims

The Specific Aims of Core C have not changed from those originally proposed.

Aim 1: Support individual projects by providing behavioral phenotyping for each project based on its objectives.

Aim 2: Support individual projects by providing electrophysiology analysis for each project based on its objectives.

Aim 3: Provide toxicological assessment of polyphenol-enriched botanical preparations in mice.

B. Studies and Results

For Aim 1 (Year 1), in collaboration with Project 1, to test the effect of select botanical treatments in the promotion of psychological resilience, we have conducted a battery of behavioral testing, including a chronic social defeat stress test, a social avoidance test to evaluate social avoidance behavior, a sucrose preference test to evaluate anhedonia and a splash test to evaluate self-neglect behavior. In collaboration with Project 2, to test the effect of select botanical treatments in the promotion of cognitive resilience to sleep deprivation stress, we conducted a battery of tests including the novel object recognition test, the Morris water maze test and the contextual fearing conditioning test. Moreover, Core C has established the flower pot method as a model for sleep deprivation that induces restricted REM sleep deprivation. We also introduced an automated sleep deprivation system from Pinnacle Inc. that is capable of inducing total sleep deprivation as well as REM sleep disruption without direct human intervention. This automated apparatus, compared to the manual “gentle handling,” can avoid direct interaction with the experimenter and can be fully standardized to avoid lab to lab variation.

For Aim 2 (Year 1), in collaboration with Project 1, to test the effect of treatment in the promotion of psychological resilience, we have conducted excitatory postsynaptic current (EPSC) recordings in the nucleus accumbens (NAc) medium spiny neurons in control mice and mice treated with select botanicals following chronic social defeat stress. In collaboration with Project 2, we measured *ex vivo* long term potentiation (LTP) and basal synaptic transmission (BST) in hippocampal slices following select botanical treatment.

For Aim 3 (Year 1), in collaboration with Projects 1 and 2, we have conducted the toxicological assessment for the mice treated with select botanicals. Specifically, we assessed the serum metabolic panel and liver function panel from mice treated with different doses of botanicals. We also performed gross mouse necropsy and complete necropsy in mouse tissues isolated from *in vivo* studies conducted by Projects 1 and 2.

C. Significance

In Year 1, we have supported the efforts of Projects 1 and 2 by performing behavioral studies and electrophysiological analyses that provide functional assessments of the molecular and cellular mechanisms by which bioactive botanicals may beneficially promote psychological resilience in the context of social defeat (Project 1) and preserve cognitive function in the context of sleep deprivation (Project 2). Core C also provided toxicological assessments for select botanical compounds tested by Projects 1 and 2, which allow the potential benefits and safety of phenolic compounds to be determined for further preclinical and clinical translational applications.

D. Future Plans

In Year 2, Core C will continue to provide state-of-the-art behavioral and electrophysiological assessments based on the goals of individual projects with the overall objective of providing support to each project that will allow the biological effects of bioactive botanicals on brain activity and function to be determined. Core C will continue to conduct toxicological assessments for all the individual botanical compounds used by each project which will provide important information on the safety of individual botanicals to be readily translated to future preclinical and clinical applications. All of the proposed studies are in line with the proposal and there is no change in the program.

E. Publications

Peer-refereed publications:

1. Zhao W, Wang J, Bi W, Ferruzzi M, Yemul S, Freire D, Mazzola P, Ho L, Dubner L, Pasinetti GM. Novel application of brain-targeting polyphenol compounds in sleep deprivation-induced cognitive dysfunction. *Neurochemistry International*. doi:10.1016/j.neuint.2015.07.023 (2015).
2. Wang J , Hodes G, Zhang HX, Zhang S , Kana V, Zhao W, Golden S, Bi WN, Menard C, Mazzola P, Xie M, Ho L, Han MH, Russo S, Pasinetti GM. Targeting inflammation and synaptic plasticity for the treatment of stress disorder and depression, 2016 (submitted).

F. Project-Generated Resources

N/A

SCIENTIFIC ABSTRACTS FROM THE POSTER SESSION AT THE SYMPOSIUM

1. **Characterization of bacterial metabolites of grape derived compounds by human gut microbiota**

Thomas Villani, Eileen Carry, Daniel Giurleo, Harna Patel, Giulio Maria Pasinetti, Lap Ho, Jeremiah Faith, Qingli Wu, and James Simon
Rutgers University and Icahn School of Medicine at Mount Sinai

In recent years, the human microbiome as a topic of study has elicited great interest among researchers studying the complex mechanisms through which food and pharmaceuticals modulate human health. Due to the complexities of microbiological metabolism, significant discussion about the impact of bacteria on pharmacokinetics and pharmacodynamics of active-compound delivery in therapeutic contexts can be found in the recent literature. In this work, we sought to identify impacts on the pharmacokinetics of grape derived products due to bacterial metabolism within the gut. Three cultures of human microflora were obtained and cultured with grape seed extract for a period of 72 hours; aliquots were taken after 24 hours. A solvent extraction method followed by derivatization for gas chromatographic analysis was developed and validated for quantification of metabolic byproducts of the polyphenolic compounds found in bacterially-fermented grape seed extract. Significant changes in the chemical profile were detected due to bacterial fermentation, supporting the idea that human gut microbiota can have significant impacts on absorption and metabolism of grape derived products.

2. **The Effects of Diet and the Microbiota in Determining Host Health**

Sean R. Llewellyn, Graham J. Britton, Eduardo Contijoch, Arthur Mortha, Miriam Merad, and Jeremiah J. Faith
Icahn School of Medicine at Mount Sinai

We have coevolved relationships with the microorganisms that colonize our body surfaces. As hosts, we provide our gut microbiota with meals that change the abundance of bacterial species and their metabolic products, which in turn can influence our physiology. In order to examine the role of dietary components on the gut microbiota, host health, and disease, we fed diets with systematically varied amounts and sources of protein, fat, carbohydrates, and fiber to mice in several disease models, including dextran sodium sulfate (DSS) colitis model and T-cell transfer colitis model, in addition to wild-type unchallenged mice. Protein and fermentable fiber had the greatest influence on health. Mice given a high protein diet had increased microbial loads and intestinal permeability, more severe disease (i.e. weight loss, pro-inflammatory cytokines, and decreased survival) in DSS colitis, as compared to a low protein diet. Fermentable fiber decreased intestinal permeability, increased the number of regulatory T cells within the lamina propria, and led to a significant decrease in disease severity for both the DSS and T-cell transfer colitis models. Furthermore, both protein and fermentable fiber act in concert to modulate health, as increases in fermentable fiber could offset the increased disease severity of a high protein diet, demonstrating that the combined influence of our dietary ingredients have a profound influence on health. The results shown demonstrate a role of macronutrients in host health and disease with the potential to test botanicals and micronutrients in our models.

3. Optogenetic analysis of polyphenol-induced resilience against sleep deprivation-associated cognitive deficits

Chad Smith, Tal Frolinger, Robert Louis, Jun Wang, Rick Dixon, Jim Simon, and Giulio Maria Pasinetti

Icahn School of Medicine at Mount Sinai, University of North Texas, and Rutgers University

Sleep deprivation (SD) is a common problem in our society and is linked to a number of physiological and cognitive co-morbidities, including memory impairments. SD disrupts the consolidation period of memory formation through downregulation of the cAMP/PKA/CREB signaling pathway, mTOR, and decreased expression of plasticity-related genes including immediate early genes (IEGs) such as c-fos. We have found that treatment with certain bioavailable bioactive phenolic metabolites (BBPMs) provides physiological resilience against SD-induced cognitive deficits, activates the CAMKII and CREB signaling pathways, and induces IEG expression. To determine the mechanism through which BBPMs provide resilience to SD in neurons activated by training, we will use c-fos-tTA transgenic mice to label memory-bearing neurons (tet-labeling) with opsin proteins expressed through a Tetracycline Response Element (TRE), a novel technology that has been used to investigate memory engrams in specific brain regions. Recently, tet-labeling has been used to generate false memories and suppress hippocampal memory engrams in the contextual fear conditioning (CFC) memory test. Ongoing experiments in our labs are testing the feasibility of this approach by injecting mice with a pan-neuronal AAV expressing eNpHR3.0 to silence hippocampal neurons bearing fear and spatial memories. In the future, c-fos-tTA mice will be injected with TRE-ChR2, treated with BBPMs and SD, and undergo footshock in context A, followed by optogenetic activation of tet-labeled neurons in an unrelated context B. Activating or silencing memory-bearing neurons will cause expression or suppression of memories, respectively, allowing for quantification of resilience to SD. Through histological analysis, we will examine the overlap of cells expressing ChR2 and c-fos, as well as CREB and other pathways to determine whether IEG-inducing BBPMs modulate these signaling pathways in memory-bearing neurons. Through histology, we will also examine the effects on signaling pathways and synaptic plasticity of optogenetically activating these populations of neurons. Through tet-labeling of specific populations of neurons, we can examine individual neuronal responses to therapy, and the responses of brain regions to optogenetic modulation.

4. Gender associated molecular responses in cognitive impairment mediated by chronic sleep deprivation

Tal Frolinger, Chad Smith, Jun Wang, and Giulio Maria Pasinetti

Icahn School of Medicine at Mount Sinai

Sleep deprivation (SD) is a common problem in our society. Chronic SD is co-morbid with a number of physiological and cognitive problems, including mental illness and memory deficits. SD is hypothesized to disrupt the consolidation period of memory formation through down regulation of cAMP/PKA/CREB, mTOR signaling pathway, immediate early genes (IEGs) and other plasticity-related genes expression. We found that certain brain bioavailable bioactive phenolic compounds (primarily flavan-3'-ols) provide resilience to acute-SD-mediated cognitive decline in male mice through activation of the CREB, CAMKIIa signaling pathways and induction of IEG expression. Most interestingly, using the novel object recognition paradigm

we found a relationship between the estrous cycle phases of low estrogen (metestrus, diestrus, estrus) and; I. Reduced cognitive performance in control females, II. Stress response in sleep deprived females, when compared to male mice in the SD model. This evidence is consistent with the fact that the estrous cycle is accompanied by hippocampal changes that affect memory consolidation, suggesting superiority in learning task performances of proestrus females compared to males. We are currently exploring gender differences in memory performance and gene expression in response to chronic SD, using the c-fos-tTATRE optogenetics system to visualize the regional distribution of signaling pathways. The study will provide an amenable system to further investigate the potential beneficial role of currently available phenolic metabolites to investigate the molecular relationship between cognitive function and changes in brain gene expression in a gender related fashion. In view of the fact that SD affects more than 70 million Americans, the social implication of this study on cognitive performance is of the foremost importance.

5. Dissecting the mechanisms by which dietary polyphenols may modulate lower back pain

Simoni Tiano, Lap Ho, Jeremiah Faith, and Giulio Maria Pasinetti
Icahn School of Medicine at Mount Sinai

Lower back pain (LBP) is a common and debilitating musculoskeletal disorder, but unfortunately, chronic LBP is often resistant to treatments. The most common cause of LBP is degeneration of intervertebral disks (IVDs) that serve to separate the vertebrae and allow for spinal motion. Key contributory pathogenic characteristics underlying discogenic LBP are IVD inflammation and structural destabilization, as well as in-growth of sensory nerve that leads to hypernociception. Based on evidence suggesting benefits of dietary polyphenols in modulating diverse medical conditions, we explored the potential of development dietary polyphenols for treating discogenic LBP. Excitedly, we observed that dietary supplementation with a select Bioactive Polyphenol-rich Dietary Preparation (BDPP) significantly reduced hypernociceptive responses in a rat model of painful disc degeneration. The majorities of orally consumed polyphenols is extensively metabolized during gastrointestinal absorption and/or post-absorptive xenobiotic metabolism and are typically accumulated in target tissues as phenolic metabolite forms. We identified 30 phenolic metabolites that are biologically available following oral BDPP administration. To gather a better understanding of how BDPP may mechanistically modulate pain associated with disc degeneration, we investigated individual phenolic metabolites for their efficacy to modulate discogenic pathogenic mechanisms using primary cell cultures generated from either nucleus pulposus (NP) or annulus fibrosus (AF), which are the two major IVD structural-functional units. In initial studies, we tested for effects of phenolic metabolites in modulating TNF α -mediated induction of key genes known to associate with IVD inflammation, extracellular matrix degradation and neuron ingrowth. We identified multiple biologically available phenolic metabolites that protected against the induction of many targeted genes. Moreover, we found two biologically available phenolic metabolites that significantly interfere with mechanisms associated with IVD destabilization, IVD inflammation and IVD abnormal sensory nerve in-growth. Ongoing investigations are exploring bioactivities of individual biologically available phenolic metabolites in primary AF cell cultures. Outcomes from our investigation support further preclinical (and eventually clinical) development of polyphenols, in either dietary or pharmacologic applications, to simultaneously target multiple

pathologic mechanisms underlying IVD degeneration and pain, which would improve the likelihood of therapeutic efficacy for treating discogenic LBP.

6. Dietary Botanicals in the Preservation of Cognitive and Psychological Resilience – Core B: Biosynthesis Component

Ronaldo J. Cavazos Jr., Jack W. Blount, Taylor D. Greer, and Richard A. Dixon
University of North Texas

The goals of the Core B Biosynthesis Component are to combine both synthetic and biosynthetic approaches to generate brain-targeted metabolites of grape-derived polyphenols. The metabolites we seek to synthesize are not commercially available. Therefore, by combining both enzymatic biosynthesis and chemical synthesis we aim to synthesize the components on large scales. Once completed, these metabolites will be used in Projects 1 & 2 with *in vitro* studies which explore the potential bioactivities and mechanisms in promoting psychological resilience (Project 1) and/or cognitive wellness (Project 2). The components will also serve as standards in the analysis of products from metabolism of dietary polyphenols through gut microbiota.