## Title

Roles for the gut microbiome in mediating the effects of diet and hypoxia on the synaptic structure and gene expression of the hippocampus



## Author

Iniguez, Alonso

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# Roles for the gut microbiome in mediating the effects of diet and hypoxia on the synaptic structure and gene expression of the hippocampus

A thesis submitted in satisfaction of the requirements for the degree Master of Science in Physiological Science

by

Alonso Iniguez

2019

ABSTRACT OF THE THESIS

Roles for the gut microbiome in mediating the effects of diet and hypoxia on the synaptic structure and gene expression of the hippocampus

by

Alonso Iniguez

Master of Science in Physiological Science

University of California, Los Angeles, 2019

Professor Elaine Yih-Nien Hsiao, Chair

Environmental factors like hypoxia and stress are important risk factors for cognitive impairment, but little is known regarding the molecular mechanisms involved. The gut microbiome is emerging as an important host-factor that mediates the effects of environmental factors on host physiologies, including brain function and behavior. As the prevalence of cognitive impairment is rapidly increasing, an active area of research is in studying how changes in the gut microbiome may impact cognitive ability in animals. Work from our laboratory has examined effects of the high fat, low carbohydrate ketogenic diet (KD) and intermittent hypoxia on the composition of the gut microbiome. Here, we highlight how the KD and hypoxia-associated microbiome impacts synaptic and transcriptomic features in the mouse hippocampus. Depletion of the microbiome in mice pre-exposed to KD and hypoxia alters global gene expression profiles in the CA3 region of the hippocampus, an area previously reported as especially vulnerable to environmental insult. Alterations in hippocampal gene expression were also observed in germ-free animals monocolonized with KD-associated bacterium, *Bilophila wadsworthia* relative to controls. The transcriptomic alterations correlated with alterations in synaptic structure in the hippocampus, as measured by altered number of excitatory synapses,

and the expression of markers for adult hippocampal neurogenesis. Altogether, these data reveal that the gut microbiome is an important mediator of the effects of the KD and hypoxia on hippocampal physiology, which could contribute to the ability of the microbiome to modify cognitive behavior.

The thesis of Alonso Iniguez is approved.

Fernando Gomez-Pinilla

David L. Glanzman

Elaine Yih-Nien Hsiao, Committee Chair

University of California, Los Angeles

2019

## DEDICATION

*Para mi familia, Florentino Iñiguez, Socorro Iñiguez, Aldo Iñiguez, y Ariel Iñiguez, su apoyo y amor es mi fortaleza.*

## TABLE OF CONTENTS

## LIST OF FIGURES

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## Introduction

*Cognitive impairment is a pressing issue caused by genetic and environmental risk factors.*

Cognitive impairment (CI) is estimated to impact 115.4 million people over the age of 60 by the year 2050[1]. As age is one of the strongest risk-factors for CI, the impending global demographic shift and lack of effective therapies and physiological markers for early diagnosis highlight the urgency to identify new interventions[2]. Several genetic polymorphisms have been implicated in the pathogenesis of conditions linked to CI, but even for heritable diseases like Alzheimer's disease (AD) and Parkinson's disease (PD), only up to 10% of cases can be attributed to genetic factors alone[3,4]. Lifestyle, nutrition, and associated comorbidities of aging like obesity and cardiovascular diseases highlight the multifactorial interactions that could contribute to the prevalence of CI. Biological aging alone does not sufficiently account for the increasing incidence of neurodegenerative disorders; as such, much of the current research examines both the intrinsic and extrinsic factors that together predispose to aging disorders. This emphasizes the necessity to understand how environmental risk factors contribute to CI and associated neurological diseases to identify etiopathogenic mechanisms that will inform novel therapeutics.

*Diet and Hypoxia are environmental risk factors for CI.*

Environmental factors, like stress and diet, can contribute to the progression of neurological disorders[5]. For example, hypoxic stress associated with high altitude, sleep apnea and pulmonary disease can lead to increased anxiety and memory deficits in humans and in animal models[6-8]. In addition, high-fat Western diets are linked to impaired hippocampal-dependent learning and memory characterized by increased blood-brain-barrier permeability, neuroinflammation, and decreased brain-derived neurotrophic factors (BDNF)[9-11]. Conversely, lifestyle interventions, like exercise, promote adult hippocampal neurogenesis and reverse the detrimental impacts of

environmentally mediated CI[12]. Therefore, it is plausible to consider that the onset and rate of progression of CI could potentially be influenced by the extent of host responses to environmental factors, like stress and diet.

*The microbiome can mediate effects of environmental risk factors, like diet, on host physiology.* The gut microbiome is a critical mediator of environmental factors on host health and disease[13]. In animal models, diet can reproducibly shape the microbial communities in the gut and the resulting immune function of the host across genotypes[13-16]. For example, Western diet regimens have been linked to increased incidence of diseases like cancer, diabetes, and autoimmune diseases, which is mediated at least in part by diet-induced alterations in the composition and function of the gut microbiota[17-19]. Beyond the known effects of the gut microbiome on peripheral immunity, the gut microbiome is increasingly known to impact the nervous system[13,20,21]. Characteristic signals of microbial dysbiosis like reduced species richness, altered metabolic processes, and increased prevalence of possible pathobionts have been correlated with neurological diseases including AD and PD[22,23]. Although direct causal relationships have not been defined, the gut microbiome bridges diverse pathways involved in host health and disease. Addressing these dietary and environmental mediated shifts of the gut microbiome can reveal insight into the impact of environmental insults on the host.

*Alterations in the gut microbiome are associated with hypoxia and with the ketogenic diet.* Unlike host genetics, the gut microbiome exhibits a high-degree of plasticity beginning at birth and the following exposure to environmental factors during postnatal development and well into adulthood[24-26]. The ability to extract resources from dietary nutrients can impart competitive advantage to some bacterial species to outcompete less adaptable bacteria. Human studies have

noted distinct microbial populations and functionality in individuals identifying as carnivore, herbivore, and omnivore[27,28]. The consumption of vegetarian protein sources can increase the prevalence of commensal *Bifidobacterium* and *Lactobacillus* while reducing pathogenic *Bacteroides* and *Clostridium*; which in turn can increase short-chain fatty acids (SCFAs)[28,29]. Shifting the available dietary nutrients can modulate the gut microbiota and its production of SCFAs, key modulators of host immune function within the gastrointestinal tract and distal organs via immunoglobulin A secretion, regulatory T-cell differentiation, and promotion of tissue barrier integrity[20,30]. For instance, a previous study reveals that the gut microbiome is necessary for mediating the effects of the high-fat:low-carbohydrate ketogenic diet (KD) on brain seizure activity. Isolation of the KD-associated bacteria and microbial transplantation into other hosts resulted in transmissible seizure-protection, demonstrating the operational impact of microbial responses to diet[31]. The impact of dietary shifts on gut microbial diversity and their associated functional characteristics like SCFA production and gene expression changes can be seen even within 24 hours of intervention[32]. Other environmental factors like hypoxia can alter gastrointestinal physiology and modify the gut microbiome due to changes in mucus barrier function[33]. The loss of stability in oxygen concentration within the intestinal lumen has been shown to disrupt the radial distribution of aerobic and anaerobic bacteria and their ability to maintain a homeostatic state[34,35]. In an oxygen-deprived environment, the gut microbiome exhibits a higher presence of *Enterobacteriaceae*, which is a signal of dysbiosis and has been associated with inflammatory diseases like colitis[36-38]. Hypobaric hypoxia is a well characterized instigator of several maladies like increased inflammation, oxidative stress, gastrointestinal permeability and CI[39]. These studies raise the important question of whether the gut microbiome contributes to the capability of environmental risk factors, like hypoxia and diet, to predispose to CI and to what extent.

*Alterations in the gut microbiome are associated with CI in animal models.*

Many studies have demonstrated that the composition and functionality of our bacterial taxa can modulate anxiety, memory, locomotor activity and social development[40-43]. The loss of microbial diversity and ensuing changes of microbiota derived signals have been associated with greater than age-expected deficiencies in language, judgement, learning and memory-- characteristic features of degenerative disorders like AD and PD[23,43-45]. In mice, depletion of the gut microbiome via antibiotic treatment (Abx) or germ-free (GF) rearing decreases spatial and working memory[46]. This makes it clear that maintaining a stable community of microbes is essential for maintaining homeostatic cognitive function. Previous work has shown that signals from PD patient-derived microbiota are sufficient to induce its hallmark physiological impairments. Conversely the restoration of *Bifidobacteria* that are reduced in the AD patient microbiome can alleviate CI and gut microbiota dysbiosis[24,43,47]. Similarly, the introduction of *Lactobacillus* and *Bifidobacterium* probiotics ameliorates impaired memory and exploratory behavior associated with a high-fat diet[26,48]. Whether the alterations in gut microbiome mediate hypoxia-induced alterations in CI is unclear. In one study, the KD ameliorated hypoxia-induced impairments in learning and memory via the promotion of BDNF expression in the hippocampus; however, the role of the role of the gut microbiome in this phenomenon was not interrogated[48]. Despite the increasing body of literature revealing microbiome regulation of cognitive behavior, the molecular, cellular, and physiological pathways by which select bacterial species of the gut contribute to CI remain elusive.

*Alterations in the gut microbiome are associated with changes in hippocampal structure and function.*

There is no defined age threshold for the onset of CI, but, interestingly, as we age, our gut shifts to a state of dysbiosis marked by an overall reduction in microbial diversity and enrichment of pathobionts[49]. These shifts have been ascribed to age-associated decreases in intestinal barrier function, but how they might mediate complex brain functions like hippocampal-dependent learning and memory is unknown[49,50]. Classically, alterations in learning and memory have been attributed to disruptions in synaptic plasticity, neuronal signaling, and molecular mechanisms largely centered in the hippocampus with projections from other brain regions[51,52]. Similarly noted disruptions associated with gastrointestinal maladies highlight the intersectionality of the gut microbiome in relationship to host and exogenous factors affecting neural dynamics in the hippocampus. But how does the gut microbiome directly or indirectly manifest changes deep in the limbic system? Suarez et al. (2018) identified how vagal afferent signaling from the intestinal tract might reach the hippocampus through secondary projections from the nucleus tractus solitarius[53]. They also noted the requirement of vagal modulation to promote the expression of BDNF and neurogenic doublecortin (DCX) signaling pathways to sustain hippocampal-dependent spatial memory performance[53,54]. Distinct signatures of microbiota derived signals can direct the differential impact on CI via alternate routes as well. For example, Abx treated or GF adult mice experience deficits in fear extinction learning associated with altered metabolite production, defective spine remodeling, and altered expression of genes of the medial prefrontal cortex, independent of the vagus nerve[55]. Other alterations in GF and Abx mice include increased volume of the CA2/CA3 regions and decreased DCX-positive cells in the dentate gyrus (DG)[46]. As hippocampal-dependent CI does not seem to be attributed to a general reduction in neuronal cells, the subtler region specific structural changes may be fundamental[56]. Within the

hippocampus, the number of synapses in the CA1 appears to be preserved, while being reduced in the CA3 and the DG of animals displaying spatial memory deficits[57]. Dietary intakes that shift the gut microbiome have been shown to mediate distinct structural effects on the hippocampus. Consumption of the high-fat diet has been noted to impair learning and memory via inhibition of PI3K/Akt pathway that can lead to synaptic dysfunction[58-60]. Diet-mediated microbial shifts can also serve as positive interventions in hippocampal-dependent deficits. Our group has previously found that the beneficial impact of the KD on epileptic seizures can be mediated by specific KD-associated bacteria that promote hippocampal GABA levels relative to glutamate[31]. The same KD significantly altered the expression of genes involved in presynaptic glutamate regulation and synaptic plasticity exclusively in the CA3 and DG[61]. Clearly, the hippocampus and gut microbiome play critical roles in the modulation of learning and memory as it pertains to environmental variables like hypoxia; however, the description of their converging pathways remains to be defined.

*Specific Aims*

These findings raise the question, do hypoxia and KD, environmental factors that modulate risk for CI also impact the composition of the gut microbiome? Also, do specific microbial taxa from the gut microbiota impact hippocampal physiology via distinct neuronal pathways that mediate CI? To answer this, I will investigate how the gut microbiome mediates the effects of the KD and hypoxia on hippocampal structure and function. To do so, I will generate cohorts of male Swiss Webster mice that consist of specific-pathogen free (SPF), Abx, GF, and GF that have been monocolonized with KD associated bacterium, *B. wadsworthia* (GF+Bilo) or *C. cocleatum* (GF+Clos) as a negative control. Using these gnotobiotic mice, I will examine synaptic structure and gene expression signatures in the CA1 and CA3 regions of the hippocampus. The differential

expression of genes pertinent to learning and memory in the CA1 and CA3 of the hippocampus will be analyzed via RNA-sequencing. Synaptic structure will be assessed using an immunohistochemical approach for excitatory and inhibitory synapses in each subfield and neurogenesis in the dentate gyrus (DG). Consistent with previous literature, the transcriptome of the CA1 subfield is not significantly impacted by the KD or the KD-associated bacterium and hypoxia gut microbiome. The CA3 subfield bears the burden of transcriptomic alterations, indicating its unique role and vulnerability in environmentally mediated CI. We also found that the prevalence of excitatory synapses across the CA1-CA3 subfields and neurogenesis in the DG were inversely affected in the Abx, GF, and GF+Clos samples, wherein the number of excitatory synapses was significantly reduced but neurogenesis increased. Altogether our work has revealed the directed effects of the microbiota and select KD-associated bacteria on hippocampal physiology that correlates with CI.

## Materials and Methods

### Mice

4-6-week old male SPF and GF wild-type Swiss Webster mice were purchased from Taconic Biosciences and bred in the UCLA Center for Health Sciences Barrier Facility and gnotobiotics facility. We established six cohorts of mice (n=6) that consisted of KD SPF Mock, KD SPF Hyp, Abx KD Hyp, GF KD Hyp, monocolonized GF+Clos and GF+Bilo. All experimental animals were fed a 6:1 ketogenic diet (93.10% fat, 6.40% protein, and 0.50% carbohydrates - Harlan Teklad TD0.7797.PWD) for 7 days before hypoxia or mock treatment. On day 8, mice were placed in a normobaric chamber and exposed to 12% oxygen intermittent hypoxia (Hyp) over 6 hour periods for 7 days. Normoxia (Mock) treated animals were placed in similar chambers and maintained at ambient oxygen level. Abx treated animals were gavaged with a solution

of vancomycin (50mg/kg), neomycin (100 mg/kg) and metronidazole (100 mg/kg) every 12 hours daily for 7 days, according to methods previously described[62]. Ampicillin (1 mg/ml) was provided *ad libitum* in drinking water. For mock treatment, mice were gavaged with sterile drinking water every 12 hours daily for 7 days. Abx-treated mice were maintained in sterile caging with sterile food and water and handled aseptically for the remainder of the experiments. GF+Clos and GF+Bilo were gavaged with $1x10^9$ CFU bacteria in 200 uL resuspension. *C. cocleatum* (DSM 1551) was obtained from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH and grown in Sweet E anaerobe broth. *B. wadsworthia* was gifted by the Devkota group at Cedars-Sinai Medical Center and grown in modified Brucella media. All microbes were cultured in anaerobic conditions. Monocolonization was confirmed using 16S rDNA sequencing. All mice were removed from their chambers on day 14 and sacrificed on day 15. All animal experiments were approved by the UCLA Animal Care and Use Committee.

**Tissue Collection and Preservation**

Hippocampal transcriptomics and synaptic morphology was explored using two separate cohorts of each mouse treatment. For transcriptomic analyses, strict macro-physiological distinctions for each tissue were followed during extraction and all tissues were dissected by same individual to ensure consistency. Tissues were immediately preserved in Trizol and stored in dry ice until RNA extraction that same day.

Brains extracted for immunohistochemical synaptic analyses were extracted using a 4% paraformaldehyde (PFA) fixation via perfusion procedure. Following perfusion, the brains were immersed into 4% PFA solution overnight and then placed into 30% sucrose media until the tissues were fully submerged to ensure proper cryoprotection of neuronal microstructures. Post

submersion in sucrose, the tissues were embedded and frozen in optimal cutting temperature compound (OCT) and stored at -80ºC.

**Hippocampal Transcriptomic Analysis**

Using an RNEasy Mini Kit (Qiagen), we extracted high-quality RNA (Average RIN: 8.9) as confirmed using the 4200 Tapestation (Agilent). RNA libraries were prepared using the QuantSeq FWD' mRNA-Seq Library Prep Kit (Lexogen) and sequenced in the Illumina HiSeq platform (1 x 65bp) by the UCLA Neuroscience Genomics Core. We used FastQC for quality control, followed by Trimmomatic to remove barcodes and any reads with an average phred score of 33. The following Trimmomatic parameters were also employed: illuminaclip:2:30:6, slidingwindow:5:30, leading:30, trailing:30, crop:65, minlen:20[63]. Parsed reads were then aligned to the mouse genome mm10 using HISAT2 to identify gene identity of reads[64]. We then obtained read counts using HTSeq-count[65]. Differential expression of genes was determined using DESeq2 in RStudio[66]. Heatmaps were constructed using the R package pheatmap, GO term enrichment analysis was conducted using DAVID, and Protein-Protein network analysis using STRING.

**Immunohistochemical Imaging**

Fixed brains were cryosectioned using a Leica CM1950 cryostat. 25um coronal sections were collected within a span of 200um and distributed between two slides beginning at the site of the hippocampal formation, determined in accordance to the Mouse P56 Coronal Reference Atlas of the Allen Institute. Slides were incubated in DAKO antigen retrieval solution (Agilent) at 90 ºC for two minutes, washed, and then blocked (0.3%PBS-T, 5% BSA, 10% normal goat serum) for one hour at room temperature. For the examination of excitatory synapses, tissues were

incubated in primary-antibody solution at 4ºC for 72 hours. After primary incubation, tissues were washed and then incubated with Alexa Fluor secondaries (1:1000) for two hours at room temperature before being washed and mounted. Primary antibody solution consisted of: anti-PSD 95 (Rabbit Polyclonal, 1:100, ThermoFisher 51-6900), anti-vGLUT 1 (Guinea Pig Polyclonal, 1:1000, Millipore AB5905), anti-vGLUT 2 (Guinea Pig Polyclonal, 1:1000, AB2251-I), anti-TuJ1 (Mouse Monoclonal, 1:200, BioLegend 801202), and anti-ZnT3 (Chicken Polyclonal, 1:500, SySy 197 006). To study inhibitory synapses and neurogenesis, tissues were incubated at 4ºC for 48 hours using the following primary antibody solution: anti-Gephyrin (Rabbit Chimeric, 1:200, SySy 147 008), anti-VGAT (Chicken Polyclonal, 1:200, SySy 131 006), and anti-DCX (Guinea Pig Polyclonal, 1:500, Millipore AB2253).

Confocal imaging for synapse analyses was performed using a Zeiss LSM 780 at 63X magnification with 1.5 zoom across 11.3 um section widths across 10 Z-stacks. Selection of synaptic puncta was strictly defined using the ImageJ plugin Puncta Analyzer with a size exclusion parameter of $0.2um^2$-$1.2um^2$ that was established by measuring co-localized puncta alongside defined axons labeled by Neuron-specific Class III B-tubulin (TuJ1)[67]. DG imaging of was performed at 20X magnification with 1.5 zoom across 8.4 um section widths across 7 Z-stacks. Quantification of DCX was performed by tracing the granule cell layers of the DG and quantitating DCX+ within enclosed area using ImageJ (NIH) particle analysis. Image optimization and orthogonal projections were performed in Zen Blue (Zeiss) and background removal was done in ImageJ.

**Statistical Analyses**

Statistical analysis of confocal images was performed using the software package Prism (GraphPad). We first averaged the number of excitatory and inhibitory across the CA1 and CA3

for each biological replicate and the percent DCX-positive area of the DG. We then performed a one-way ANOCA with multiple comparisons and Tukey corrections. Significance levels are indicated by *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001. Notable non-significant (and non-near significant) differences are indicated in the figures by ‘‘n.s.’’.

## Results

### The Ketogenic Diet and Hypoxia-Associated Gut Microbiome Alters Gene Expression in the Hippocampus

To evaluate the effect of the KD and hypoxia mediated gut alterations on the transcriptomics of the CA1 and CA3 hippocampal subfields, we performed RNA sequencing on hippocampal tissues from gnotobiotic mice. First, we compared the expression levels of each subfield isolating the effects of hypoxia (SPF KD Mock vs SPF KD Hyp) and the gut microbiome (SPF KD Hyp vs Abx KD Hyp & GF KD Hyp) (**Fig. 1)**. Our approach yielded more than 6 million reads per sample and annotated 13,728 genes in CA1 and 9,867 genes in CA3. Aligning with previous research, we did not observe any differential gene expression in response to hypoxia in either of the subfields 24 hours after the last hypoxic exposure[68]. The CA1 subfield did not exhibit any significantly differentiated genes that could be attributed to microbiome changes induced by KD after hypoxia treatment (**Fig.2**). It has been previously noted that the different subfields of the hippocampus suffer different degrees of neurodegeneration due to hypoxia with the CA3 bearing the brunt of the impact[68]. Other researchers have also noted that the CA1 subfield is exempted from KD-mediated effects on synapse-related gene expression in aging animal models[61].

In contrast to CA1, the CA3 subfield displayed 787 significant differentially expressed genes. 137 of those genes were mutually affected by Abx KD Hyp and GF KD Hyp in comparison to

SPK KD Hyp (**Fig. 3**). This intersection of differentially expressed genes in both the Abx and GF highlight molecular pathways modulated by the KD and Hyp-induced gut microbiome. Of the 137 mutually altered genes, 53 of them were upregulated and mapped to myelination, ion transport, and axon guidance pathways; the remaining 84 downregulated genes highlighted chemical synaptic transmission and eating behavior pathways (**Fig. 4**). Furthermore, protein network analysis of the upregulated genes identified neuronal activity and cell structure pathways, while the downregulated protein interactions highlighted protein binding, lipid and fatty acid metabolism, and hormone signaling (**Fig. 4)**.

Second, we compared gene expression in each subfield of monocolonized GF+Bilo and GF+Clos mice. Our group has previously noted that the prevalence of *B. wadsworthia* increases in GF mice transplanted with KD SPF Hyp microbiota relative to KD SPF Mock, while *C. cocleatum* is significantly enriched in KD SPF Mock microbiota transplants relative to KD SPF Hyp. Using the same library and sequencing preparation, we yielded more than 12 million reads per sample and annotated 15,612 genes in CA1 and 18,913 genes in CA3. We found 4 genes that were significantly differentially expressed in CA1 in GF+Bilo relative to GF+Clos: *Pank4*, 4933429O19Rik, and *Msr1* were upregulated and *Lhfpl5* was downregulated (**Fig. 5)**. In CA3, 12 genes were significantly up-regulated and 21 were down-regulated in GF+Bilo relative to GF+Clos. No genes were significantly differentiated in both subfields (**Fig. 6**). Functional Gene Ontology (GO) annotation of genes significantly differentiated in CA3 highlighted peptidyl-prolyl cis-trans isomerase activity, negative regulation of transcription from RNA polymerase II promoter, outflow tract morphogenesis, response to mechanical stimulus and neuromuscular junction (**Fig. 6)**. The contrast in number of differentially expressed genes between subfields suggests that dysregulation of CA3 may be responsible for the observed behavioral and

electrophysiological deficits mediated by the gut microbiome. In alignment with our expectations, these impairments highlighted alterations in expression of genes related to synaptic transmission and neuronal activity protein networks.

**The Ketogenic Diet and Hypoxia-Associated Gut Microbiome Alters Synaptic Morphology and Neurogenesis in the Hippocampus**

Our transcriptomic analysis revealed that the KD and Hyp associated gut microbiome altered the expression of genes associated with synaptic transmission and neuronal activity in the CA3 of the hippocampus. Although the precise mechanisms by which the gut microbiome modulates gene expression in the brain remains unclear, genetic correlates to cognitive performance have been described previously. The altered expression of calbindin, CamKs, and Ras and Rho GTPases has been associated with alterations in memory performance, dendritic spine integrity, and synaptic plasticity. To determine whether the gene expression changes correlate with morphological alterations in synaptic structure, we performed immunohistochemical staining for synaptic proteins. The CA3-CA1 intersection, the Schaffer collateral, is an essential formation for memory consolidation and retrieval through activity-dependent plasticity. We identified each hippocampal subfield by using the marker ZnT-3, which is expressed abundantly in the mossy fibers of the CA3[69] (**Fig. 7)**.

There was no difference in the number of labeled excitatory or inhibitory synapses between the CA3, CA3-CA1, and CA1 regions of the hippocampus across cohorts. Averaging the number of synapses across the CA3-CA1 intersection, there is a significant decrease in excitatory synapses in Abx KD Hyp, GF KD Hyp, and GF+Clos relative to SPF KD Hyp and GF+Bilo (**Fig. 10**). This suggests that depletion of the gut microbiome impacts excitatory synapses across the

hippocampus, even though transcriptional alterations were largely localized to the CA3 as opposed to the CA1. There was no significant difference in the number of inhibitory synapses averaged across the subfields (**Fig. 10**).

The DG is the input region of the hippocampus that plays a critical role in learning and memory and is one of the few brain regions with evidence of adult neurogenesis[70]. We used particle analysis to quantify the area of positive labeling of neurogenesis markers relative to the total area of the DG. We uncovered a significant increase in neurogenesis in Abx KD Hyp and GF KD Hyp relative to KD SPF Mock, KD SPF Hyp, and GF+Bilo (**Fig. 12**). We also noted that the GF+Clos cohort demonstrates a significant increase relative to GF+Bilo (**Fig. 12**). It is interesting to note the inverse relationship between excitatory synapses across the CA3-CA1 subfields and neurogenesis in the DG.

**Discussion**

A variety of etiologies for CI have been proposed defining how genetic risk factors and environmental exposures can intersect to manifest behavioral alterations. Both hypoxia and KD interact synergistically to impair hippocampal-dependent learning and memory associated with precocious aging pathologies as our group has previously noted the measurable behavioral detriments imposed by the transfer of KD and hypoxia gut microbes. Herein we have identified the KD and hypoxia-associated gut microbiome as a key factor in the multifactorial network of CI-related alterations in hippocampal transcriptomics and synaptic physiology in wild-type Swiss Webster mice.

The hippocampus is highly susceptible to changes in the environment in both health and disease paradigms with each subfield ascribed to unique phenotypes. In general terms, the CA3 is necessary for rapid pattern separation and acquisition, whereas the CA1 is fundamentally involved in the consolidation process by producing effective retrieval cues[71,72]. As the hippocampus is a transitory structure that relays newly encoded hippocampal-dependent memories to their permanent storage sites in neocortical regions, how distinct environmental exposures affect this process must be acutely dissected in order to develop effective interventions beneficial to the several disorders involving hippocampal disruption, such as AD[73].

We noted that the burden of the KD and hypoxia associated gut microbiome is on the CA3. The absence of the KD and hypoxia gut microbiome via Abx treatment or GF rearing highlighted 53 genes that were significantly upregulated and 84 genes downregulated. Only 6 genes that were downregulated displayed a log-fold change greater than 2: *Lgr5, Cartpt, Baiap3, Klhl1, Calb2,* and *Pmch*. The products of the prepropeptides *Pmch* and *Cartpt* have been noted to improve memory retention post-training and confer neuronal and behavioral benefits in mice with αβ plaques after exogenous treatment into animal models[74,75]. Deficiency in the expression of *Calb2* and *Klhl1* has been associated with reduced synapsin-labeled puncta, dendritic atrophy, and detrimental effects on purkinje cell activity[76,77], whereas decreased expression of *Baiap3* and *Lgr5* inhibits neuron development and functions[78]. Other environmental factors like early life stress have been shown to lead to persistent changes in gene expression in CA3 neurons well into adulthood[79]. Similarly, the CA3 subfield has been observed to bear the brunt of hypoxia impacts with the highest density of ROS and with it, the impact of the cognitive impairment[68].

Monocolonization with *B. wadsworthia* and *C. cocleatum* highlighted different outcomes on the CA3 and CA1 subfields. Most of the effects were seen in the CA3; however, the CA1 displayed 3 genes that were upregulated and 1 downregulated in GF+Bilo in relation to GF+Clos. As the GF+Bilo was developed from a KD and hypoxia-associated bacterium, its results may highlight the mediators of CI. Among the upregulated genes, the regulation of *Msr1* has been implicated with AD modifications in mice[80]. In the CA3, significantly upregulated genes included *Kcnn3*, a regulator of neuronal excitability via calcium-potassium channels, and *Arghap6*, an effector of actin-polymerization that could impact spine-remodeling. Genes that were downregulated in the CA3 included, *Filip1L,* regulator of antiangiogenic activity that can inhibit cell proliferation, migration, and apoptosis, and *P4ha3* which is involved in oxidoreductase activity via the incorporation or reduction of molecular oxygen. Altogether our results agree with previous observations of the CA3 susceptibility to environmental factors, but much remains to be defined in the ongoing effort to identify the distinct responses of each hippocampal subfield to CI-related factors and link these to phenotypic behavioral impairment[81]. As each subfield contributes to different aspects of learning and memory, such as pattern separation, consolidation, and retrieval, they clearly display different vulnerabilities that must understood.

The differential impacts of the KD on synapse-related gene expression between the CA3 and CA1 has been previously described, but how these relay their effects on synaptic morphology is unknown[61]. Interestingly, despite the differences in the expression of genes there were no differences in the number of synapses expressed between the subfields. But there was a significant decrease in the average of excitatory synapses in the absence or depletion of the gut microbiome and the GF+Clos cohort. This was of particular interest to our group, as we had previously noted that the Abx KD Hyp, GF KD Hyp, and GF+Clos cohorts demonstrated

improved cognitive performance compared to conventionally colonized and Bilophila-colonized mice. With no significant impact on inhibitory synapses, these results suggest that aberrations in the neuronal excitation process may be responsible for the observed CI. Multiple triggers like mitochondrial dysfunction, calcium overload, and oxidative stress can induce deleterious signaling leading to excitotocity and neuronal death. Death of neurons by excessive stimulation has been implicated in CI-related ailments like PD and AD[82].

The maintenance of neurons is essential for healthy cognitive behavior. Adult hippocampal neurogenesis continues well into adulthood, but sharp decreases in neurogenesis have been linked to CI and AD[83]. The number of immature neurons labeled by DCX in the DG was reduced in the Abx KD Hyp, GF KD Hyp, and GF+Clos cohorts as opposed to conventionally colonized and Bilophila-colonized mice. GF+Bilo demonstrated the lowest levels of neurogenesis in relation to the other cohorts. This suggests that elevated excitatory synapses across the CA1-CA3 coupled with reduced neurogenesis in the DG could contribute to microbiota-induced effects on cognitive impairment.

Overall, our exploration of how the gut microbiome mediates the effects of the KD and hypoxia on host brain and behavior reveals that the microbiome impacts synaptic structures and transcriptomic signatures within the hippocampus. In accordance with previous research, our work highlighted the high vulnerability of the CA3 subfield to environmental perturbation and suggests that aberrant neuronal excitability could contribute to CI. Additionally, we revealed that neurogenesis in the DG can be modified by monocolonization with particular bacteria, *C. cocleatum* and *B. wadsworthia*. This further highlights the importance of studying the gut microbiome as a regulator of CI, toward revealing a possible point of clinical intervention.

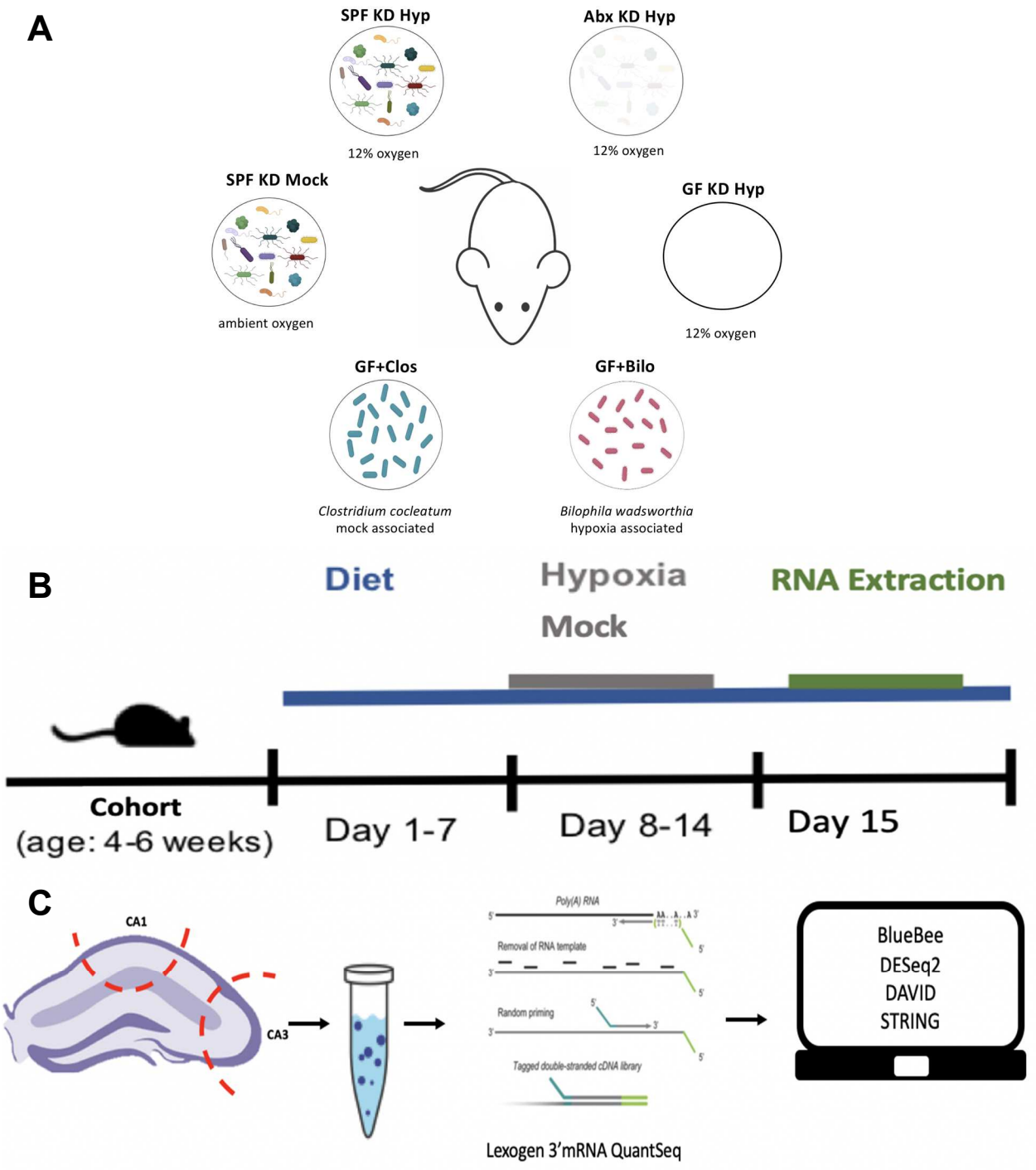


**Figure 1. Experimental approach to study the effects of KD and hypoxia.** A) Six cohorts of male Swiss Websters (n=6) were established to detail how the KD microbiome affects the host response to hypoxia. KD SPF Mock, KD SPF Hyp, Abx KD Hyp, and GF KD Hyp. The GF+Bilo (*B. wadsworthia*) and GF+Clos (*C. cocleatum*) cohorts were monocolonized with their respective bacterium to note possible specific mediators of the KD effect. B) Cohorts were sacrificed on day 15 in order to parallel the beginning of behavioral assays. C) Morphometric extractions of the CA3 and CA1 subfields remained consistent and RNA preserved in within 24 hours. The average RNA concentration was 50ng/uL and the average RIN score was 8.9. Library preparation was performed using the QuantSeq 3' mRNA-Seq Library Prep Kit FWD for Illumina (Lexogen). Transcripts were aligned and referenced using the BlueBee genomics analysis platform, differential expression calculated with DESeq2, pathway analysis using DAVID, and protein-protein interactions using STRING.

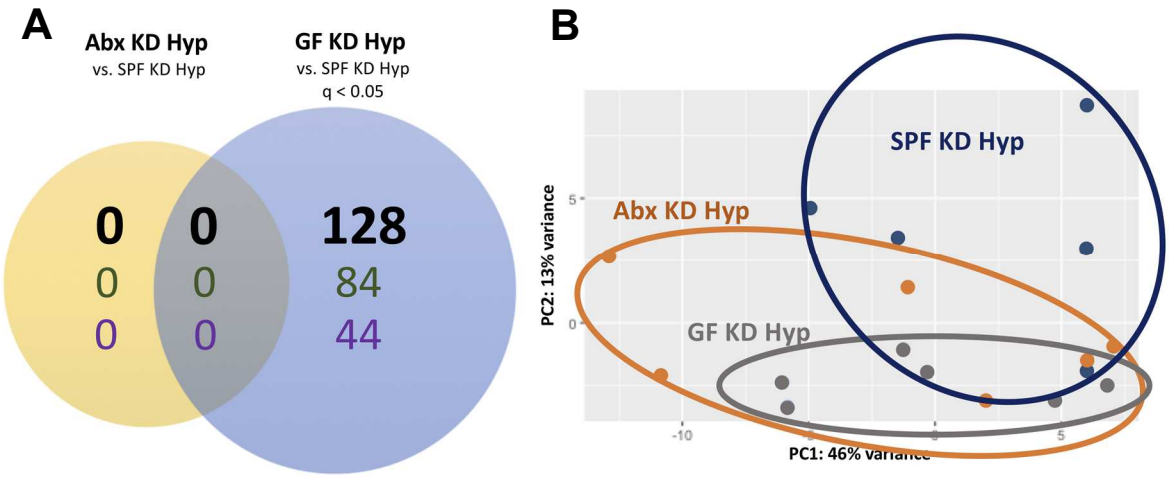


**Figure 2. Depletion of the KD- and hypoxia-associated microbiome does not impact gene expression in the CA1 region of the hippocampus.** A) Differentially expressed genes ($q < 0.05$) in the CA1 subfield of the hippocampus from Abx KD Hyp vs SPF KD Hyp and GF KD Hyp vs SPF KD Hyp (n=5-6). Bolded numbers represent total differentially expressed genes, numbers in green are upregulated genes, numbers in magenta are downregulated genes. B) Principal Coordinate Analysis of all transcripts revealed low variance and overlap between clusters.

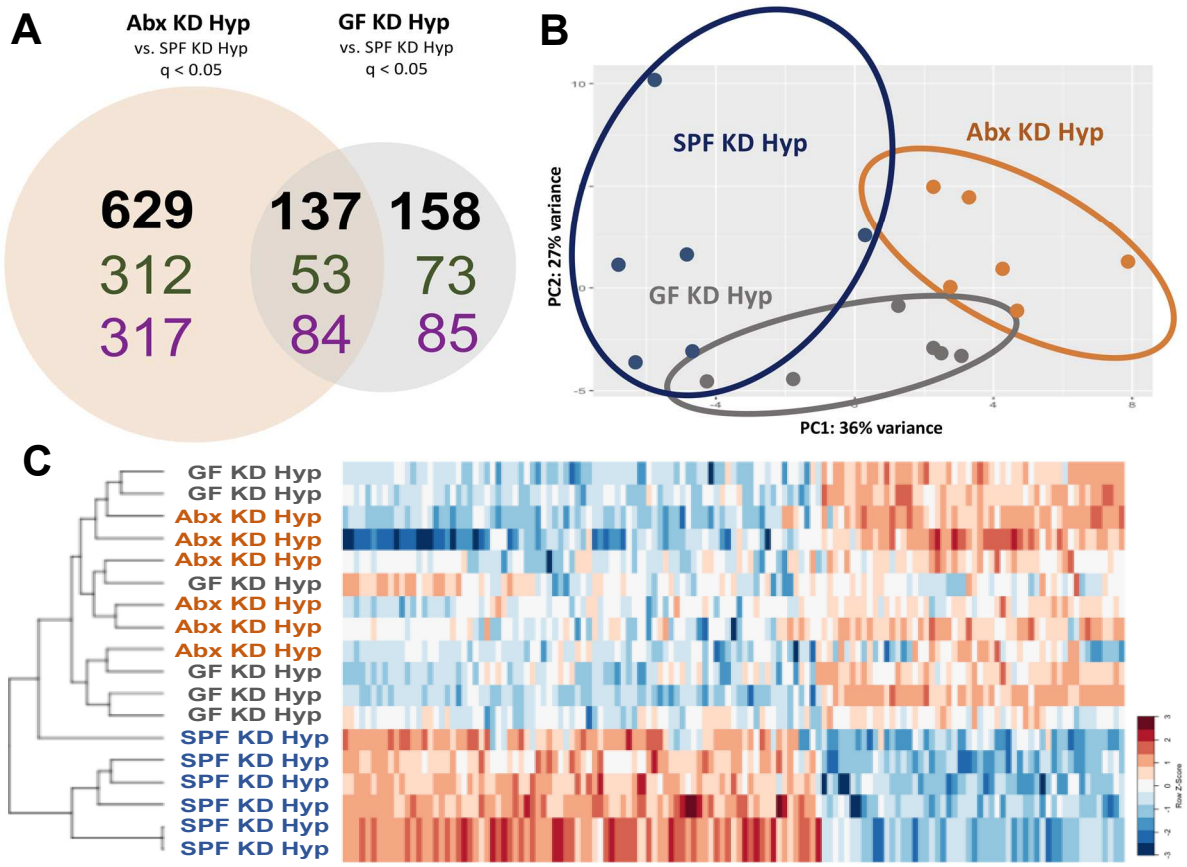


**Figure 3. Depletion of the KD- and hypoxia-associated microbiome alters gene expression in the CA3 region of the hippocampus.** A) Differentially expressed genes (q < 0.05) in the CA3 subfield of the hippocampus from Abx KD Hyp vs SPF KD Hyp and GF KD Hyp vs SPF KD Hyp (n=5-6). Numbers at the intersection of the venn diagram represent the genes that were mutually differentiated by GF KD Hyp and Abx KD Hyp vs SPF KD Hyp. Bolded numbers represent total differentially expressed genes, numbers in green are upregulated genes, numbers in magenta are downregulated genes. B) Principal Coordinate Analysis of all transcripts reveals cluster differentiation across the groups. C) Heatmap of the mutually differentiated 137 genes by Abx KD Hyp and GF KD Hyp vs SPF KD Hyp.

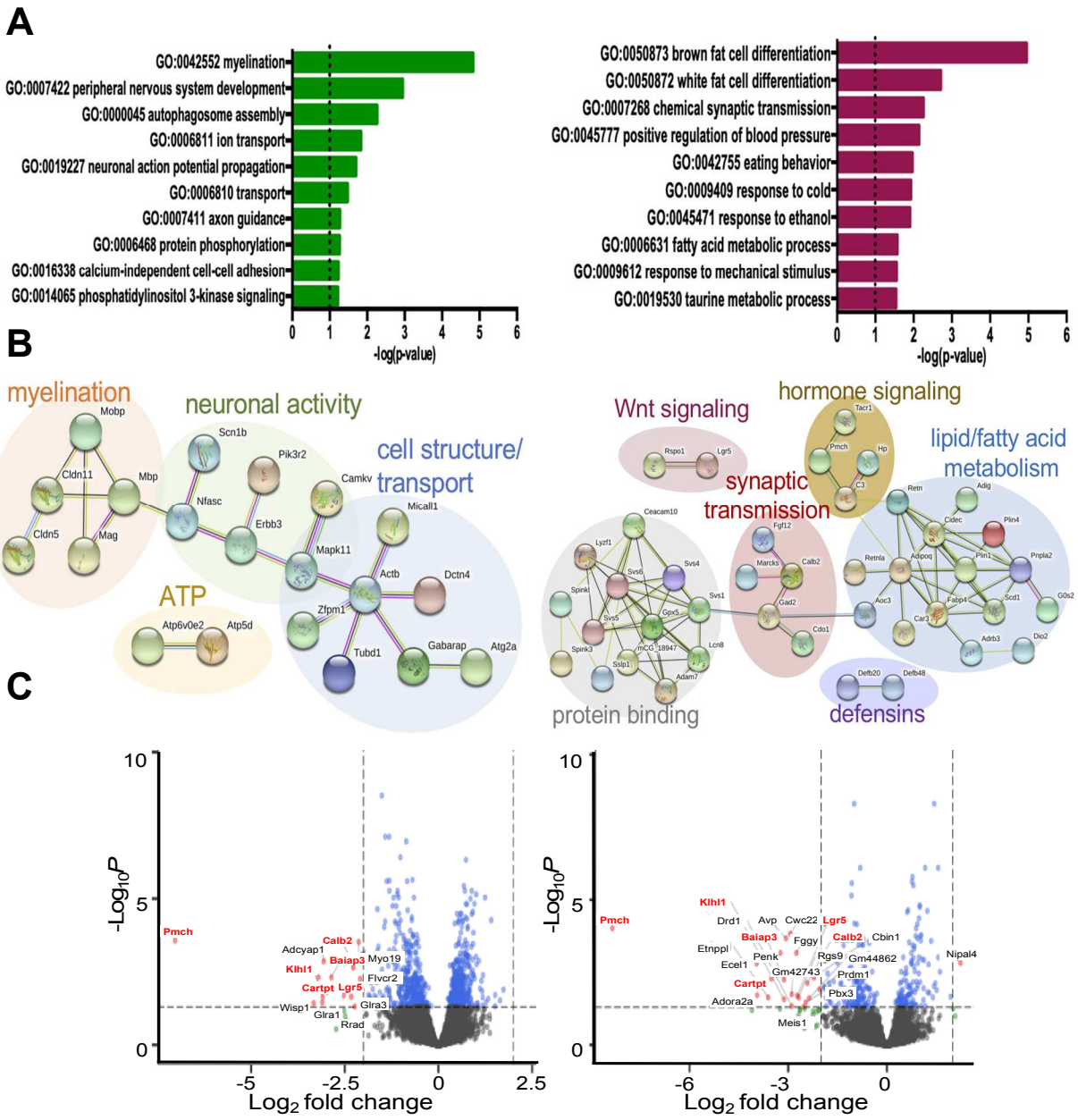


**Figure 4. Depletion of the KD- and hypoxia-associated microbiome alters the expression of genes mapping to pathways for neurotransmission.** A) Top 10 pathways from GO-Term enrichment analysis of the 53 upregulated genes (green) and 84 downregulated genes (magenta) by microbiome depletion in both Abx and GF in KD Hyp mice. B) Protein Network Analysis of 53 upregulated genes (left) and 84 downregulated genes (right) from Abx KD Hyp and GF KD Hyp mice compared to SPF KD Hyp controls. C) Volcano plots of pairwise comparisons labeling differentially expressed genes (LFC2 > 2) in Abx KD Hyp mice (left) and GF KD Hyp mice (right) compared to SPF controls. Differentially expressed genes shared by both Abx and GF conditions are labeled in red ($q < 0.05$).

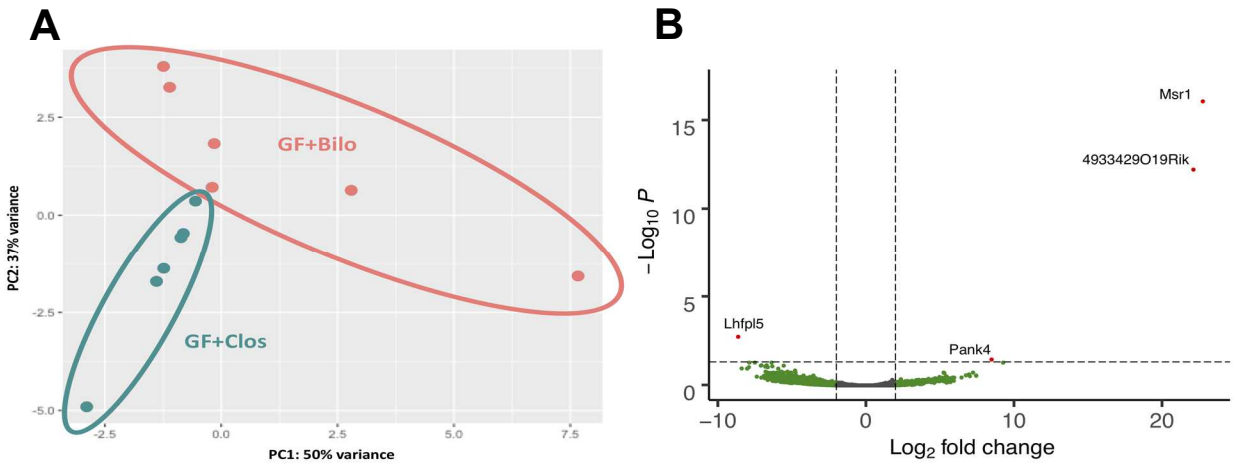


**Figure 5. Monocolonization of mice with B. wadsworthia or C. cocleatum has minimal effects on gene expression in the CA1 region of the hippocampus.** A) Principal Coordinate Analysis of all transcripts reveals differences in expression between GF+Bilo and GF+Clos with minimal overlap. B) Volcano plot labeling differentially expressed genes (LFC2 > 2) in GF+Bilo relative to GF+Clos.

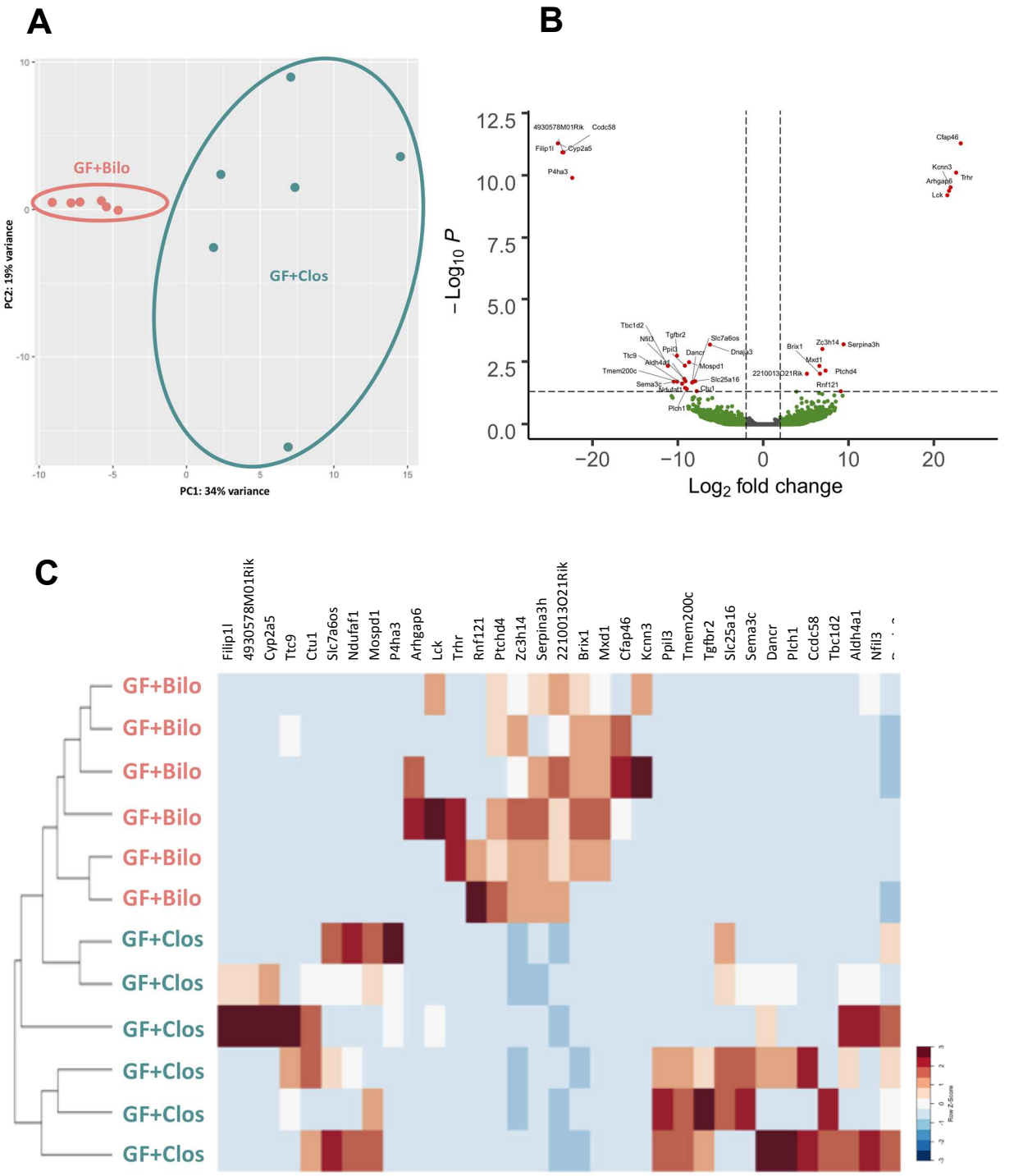


**Figure 6. Monocolonization of mice with *B. wadsworthia* or *C. cocleatum* alters gene expression in the CA3 region he hippocampus.** A) Principal Coordinate Analysis of all transcripts reveals differences in expression between GF+Bilo and GF+Clos clear clusters of differentiation. B) Volcano plot labeling differentially expressed genes (LFC2 > 2) in GF+Bilo relative to GF+Clos. C) Heatmap of all genes that were significantly differentiated in GF+Bilo and GF+Clos. D) Top pathways from GO-Term enrichment analysis of all genes significantly altered.

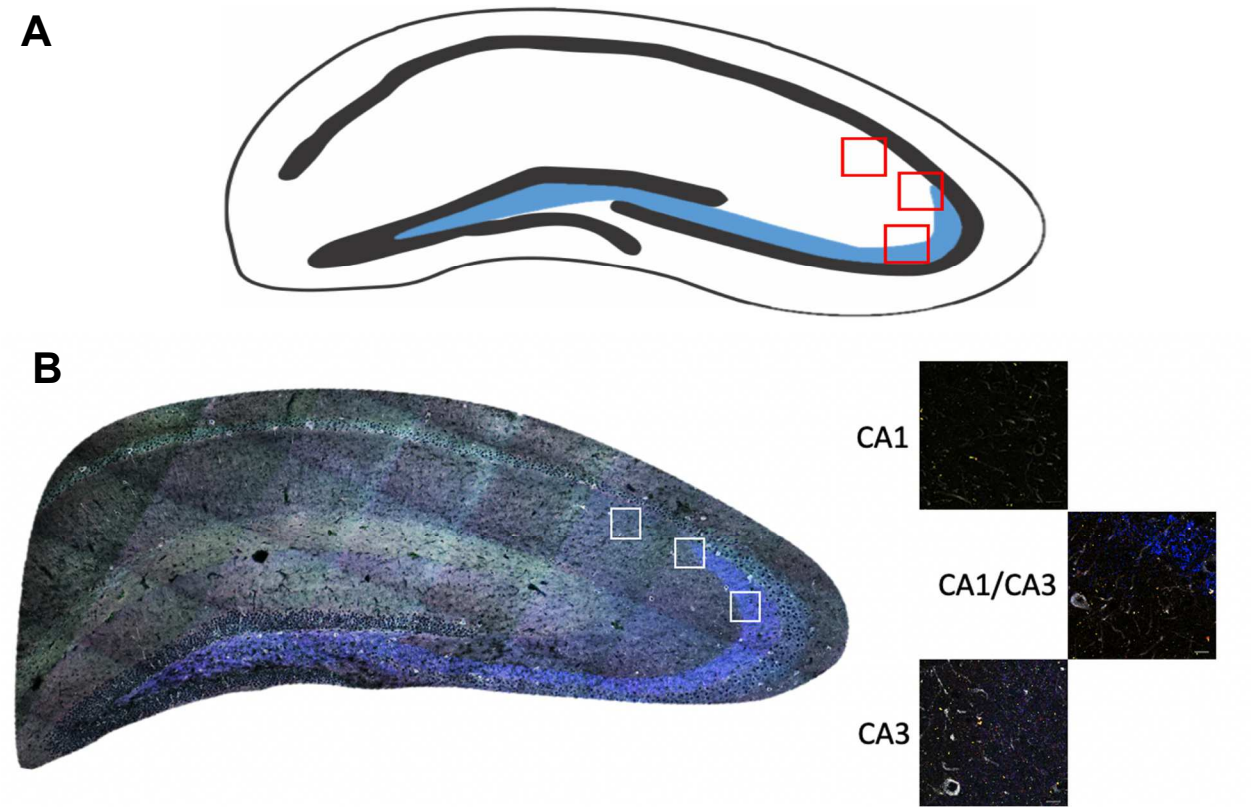


**Figure 7**. **Identification of morphological intersection of CA3-CA1 regions.** A) Confident identification of the CA3/CA1 intersection is feasible with the expression of Znt-3 in the mossy fibers. Quantification of excitatory synapses is performed in the stratum radiatum, 10um below the stratum pyramidale. Total range of measurements spans approximately 500um with 100um intersects. B) Representative image of whole hippocampus tiled from 20x 283.4um$^2$ scans (left). Representative images of CA1, CA1/CA3, CA3 sections labeled for excitatory synapses.

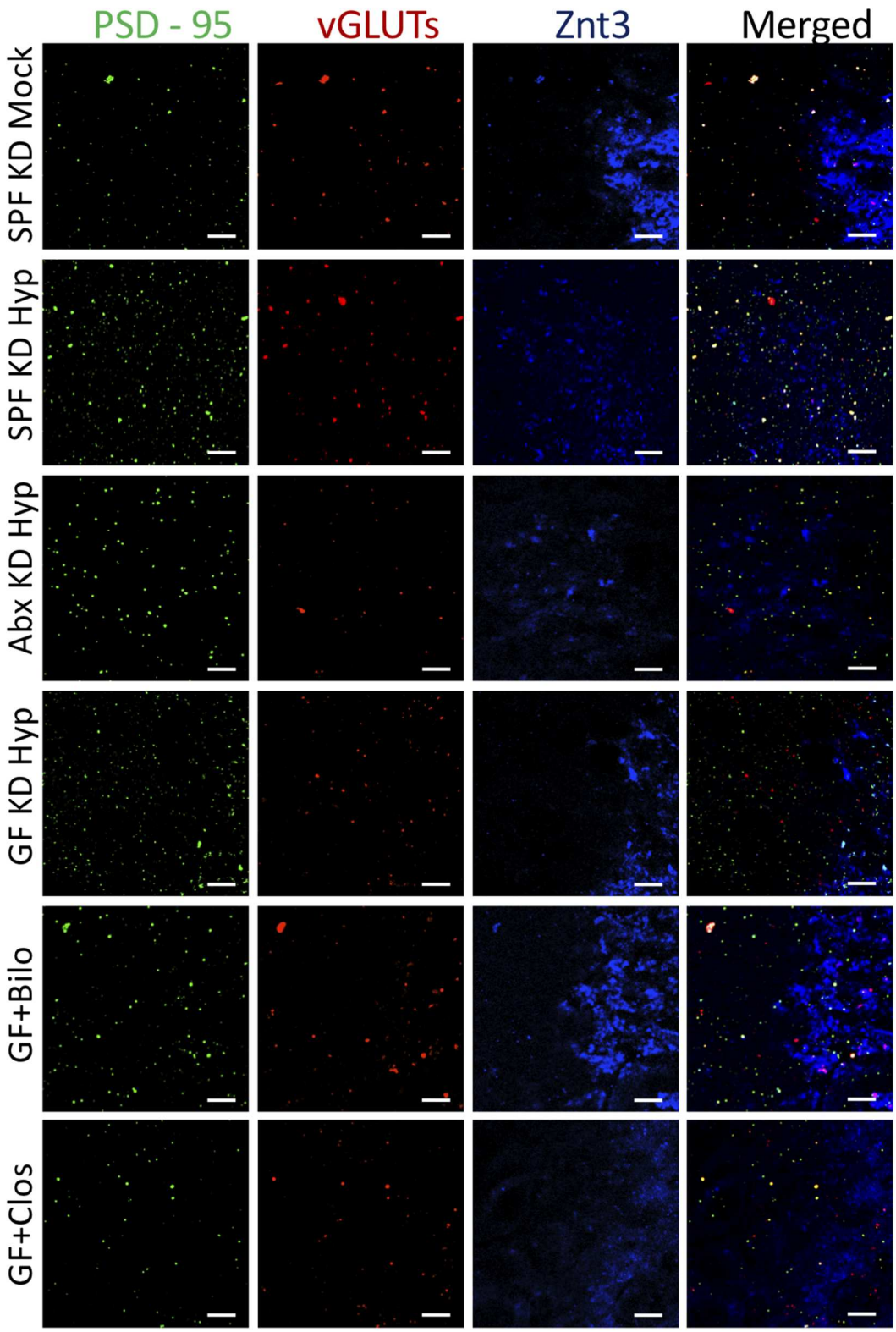


**Figure 8. Excitatory synapses across the Schaffer Collateral.** Immunohistochemical visualization of excitatory synapses at 63x magnification using PSD-95 (green) and vGLUTs (red). The CA3-CA1 intersection was identified by finding the edge of Znt-3 expression. Co-localization was measured by the ImageJ plugin Puncta Analyzer using $0.2um^2$-$1.2um^2$ size discretion calculated by measuring excitatory synapses alongside labeled axons (TUJ1).

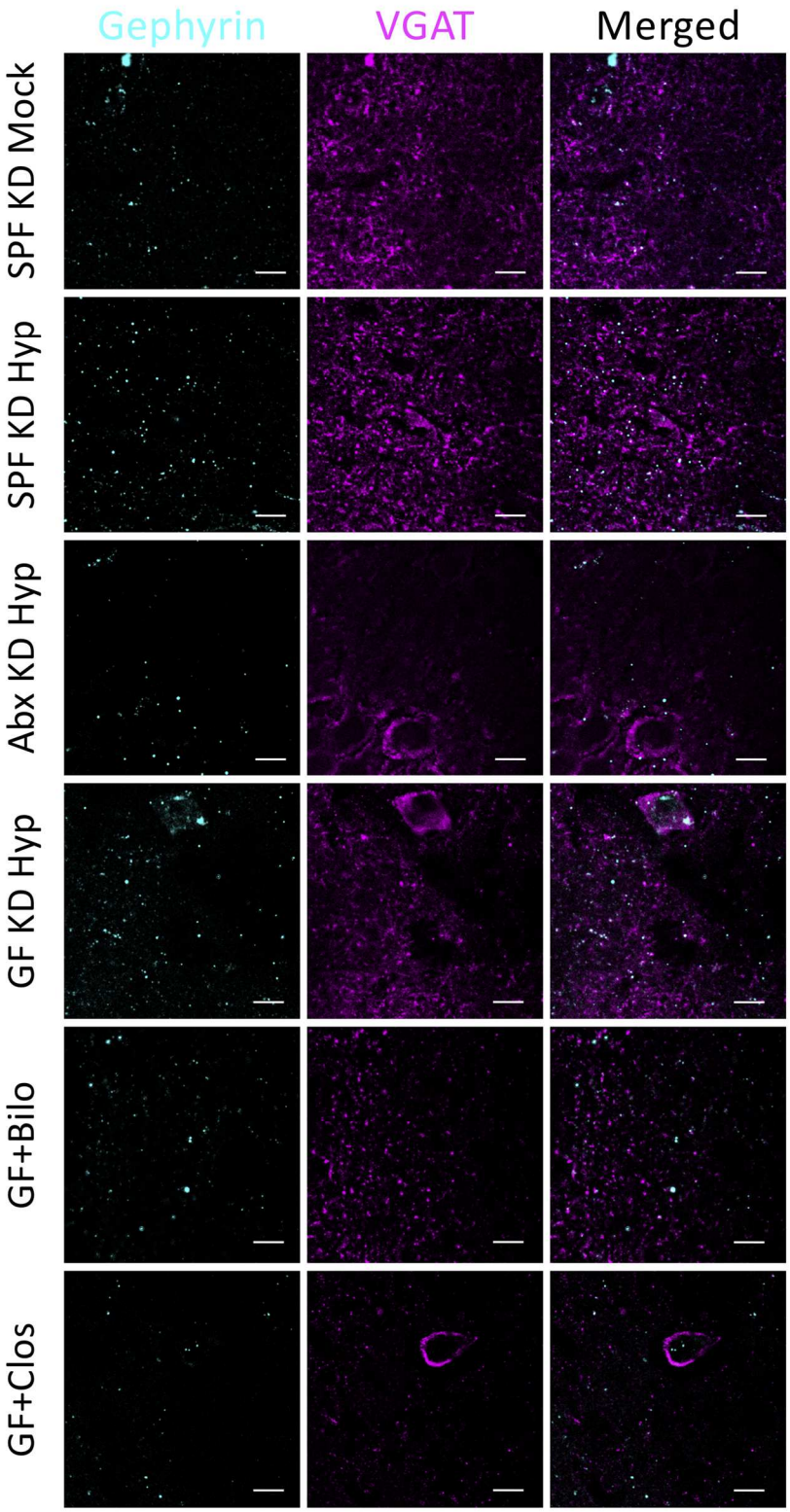


**Figure 9. Inhibitory synapses across the Schaffer Collateral.** Immunohistochemical visualization of inhibitory synapses at 63x magnification using Gephyrin (cyan) and VGAT (magenta). The number of inhibitory synapses were measured by the ImageJ plugin Puncta Analyzer using $0.2um^2$-$1.2um^2$ size.

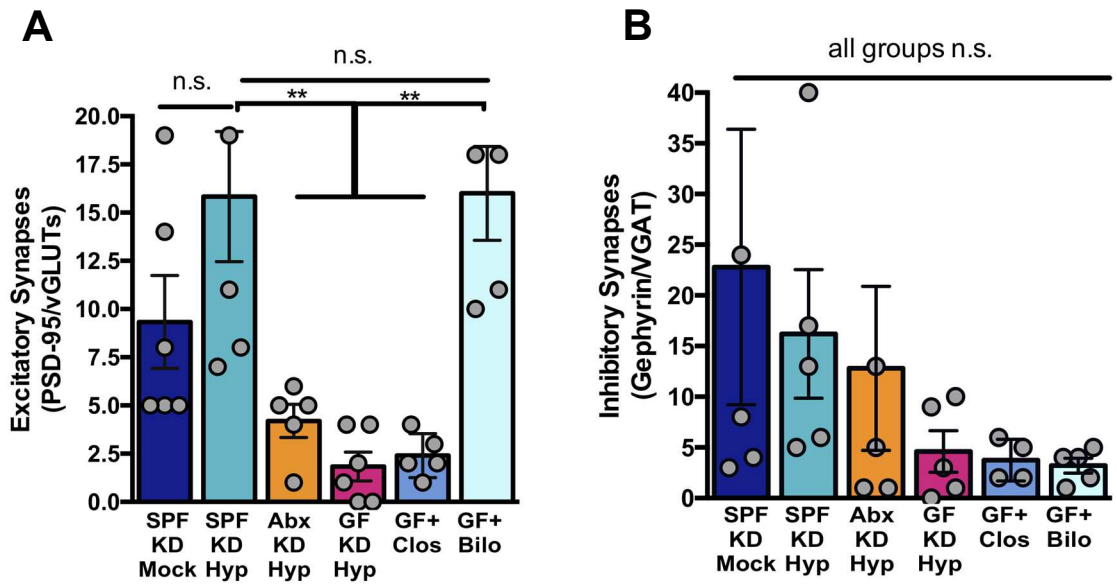


**Figure 10. Average number of excitatory synapses and inhibitory synapses across the CA3-CA1 are differentially impacted by the KD- and hypoxia-associated gut microbiome.** A) There is a significant decrease in the number of excitatory synapses in Abx KD Hyp, GF KD Hyp, and GF+Clos across all three sites, CA3, CA3-CA1, CA1. B) Inhibitory synapses are not significantly impacted by the KD and Hyp gut microbiome.

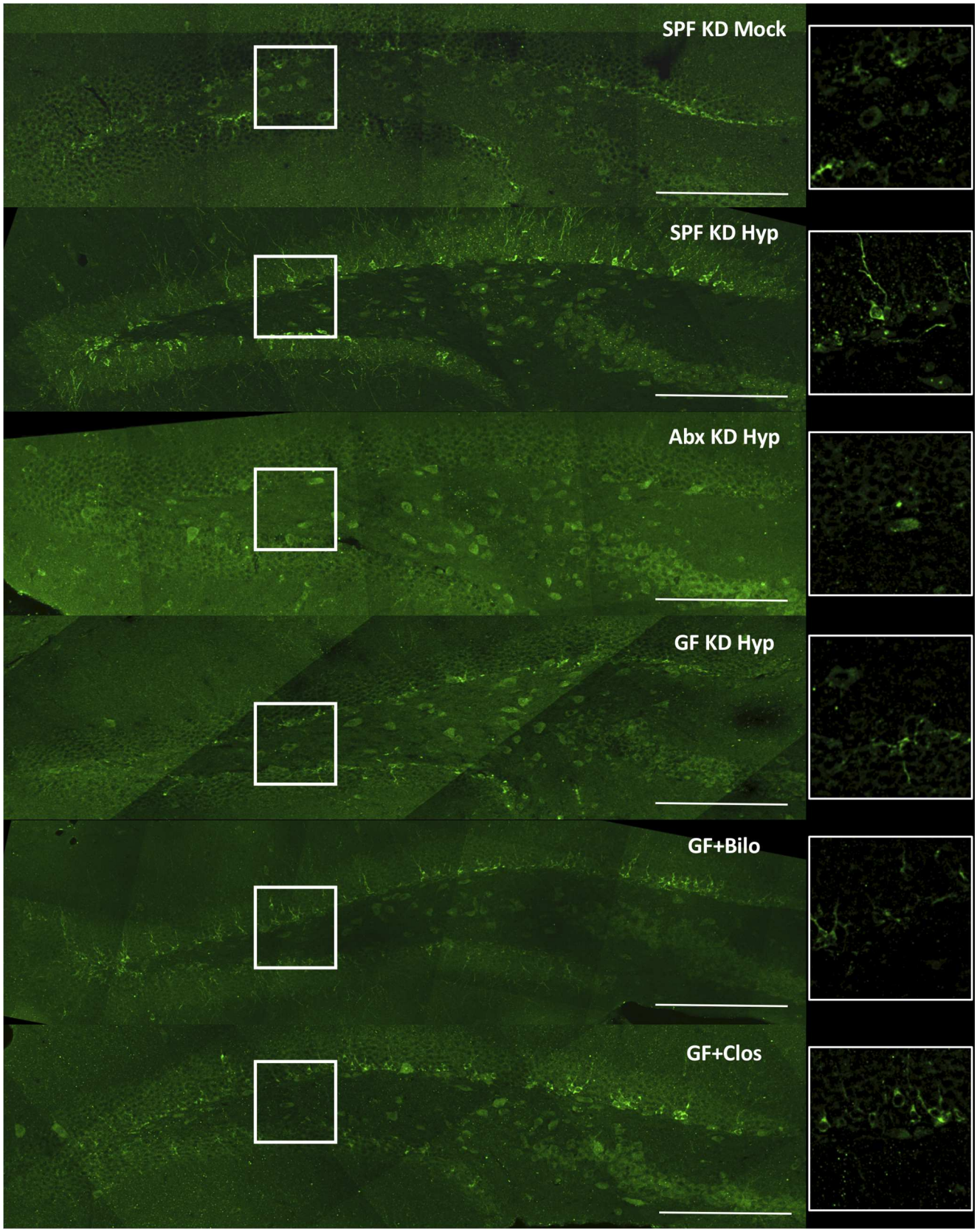


**Figure 11. Adult hippocampal neurogenesis in the dentate gyrus (DG).** Immunohistochemical visualization of DCX+ cells at 20X magnification in the DG as defined by the granule cell layer.

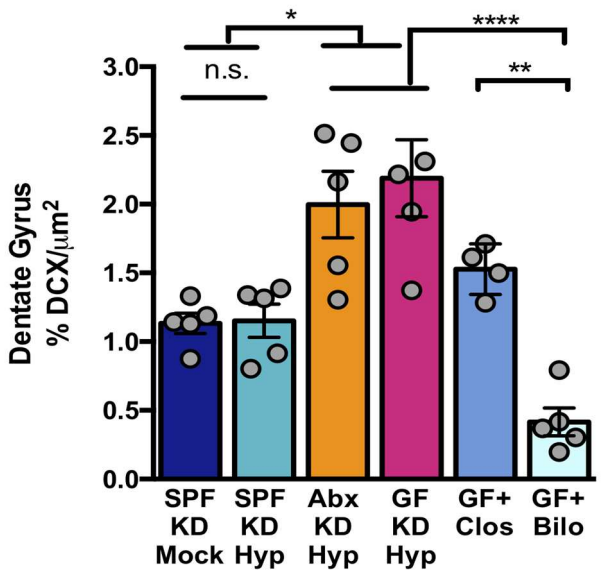


**Figure 12. Neurogenesis in the DG is altered by the KD and hypoxia associated gut microbiome.** The absence or depletion of the gut microbiome as well as *C. cocleatum* increase the level of neurogenesis in the DG relative to KD SPF Mock, KD SPF Hyp, and GF+Bilo.

**Bibliography**


1 Dementia: a public health priority. (World Health Organization and Alzheimer's Disease International, 2012).

2 World Population Ageing. (United Nations, 2009).

3 Guerreiro, R. J., Gustafson, D. R. & Hardy, J. The genetic architecture of Alzheimer's disease: beyond APP, PSENs and APOE. *Neurobiol Aging* **33**, 437-456, doi:10.1016/j.neurobiolaging.2010.03.025 (2012).

4 Nalls, M. A. *et al.* Large-scale meta-analysis of genome-wide association data identifies six new risk loci for Parkinson's disease. *Nat Genet* **46**, 989-993, doi:10.1038/ng.3043 (2014).

5 Brown, R. C., Lockwood, A. H. & Sonawane, B. R. Neurodegenerative diseases: an overview of environmental risk factors. *Environ Health Perspect* **113**, 1250-1256, doi:10.1289/ehp.7567 (2005).

6 Asmaro, D., Mayall, J. & Ferguson, S. Cognition at altitude: impairment in executive and memory processes under hypoxic conditions. *Aviat Space Environ Med* **84**, 1159-1165, doi:10.3357/asem.3661.2013 (2013).

7 Dewan, N. A., Nieto, F. J. & Somers, V. K. Intermittent hypoxemia and OSA: implications for comorbidities. *Chest* **147**, 266-274, doi:10.1378/chest.14-0500 (2015).

8 Kirkil, G. *et al.* The evaluation of cognitive functions with P300 test for chronic obstructive pulmonary disease patients in attack and stable period. *Clin Neurol Neurosurg* **109**, 553-560, doi:10.1016/j.clineuro.2007.03.013 (2007).

9 Hargrave, S. L., Davidson, T. L., Zheng, W. & Kinzig, K. P. Western diets induce blood-brain barrier leakage and alter spatial strategies in rats. *Behav Neurosci* **130**, 123-135, doi:10.1037/bne0000110 (2016).

10 Molteni, R., Barnard, R. J., Ying, Z., Roberts, C. K. & Gomez-Pinilla, F. A high-fat, refined sugar diet reduces hippocampal brain-derived neurotrophic factor, neuronal plasticity, and learning. *Neuroscience* **112**, 803-814, doi:10.1016/s0306-4522(02)00123-9 (2002).

11 Rutkowsky, J. M. *et al.* Reduced cognitive function, increased blood-brain-barrier transport and inflammatory responses, and altered brain metabolites in LDLr -/-and C57BL/6 mice fed a western diet. *PLoS One* **13**, e0191909, doi:10.1371/journal.pone.0191909 (2018).

12 Choi, S. H. *et al.* Combined adult neurogenesis and BDNF mimic exercise effects on cognition in an Alzheimer's mouse model. *Science* **361**, doi:10.1126/science.aan8821 (2018).

13 Round, J. L. & Palm, N. W. Causal effects of the microbiota on immune-mediated diseases. *Sci Immunol* **3**, doi:10.1126/sciimmunol.aao1603 (2018).

14 Carmody, R. N. *et al.* Diet dominates host genotype in shaping the murine gut microbiota. *Cell Host Microbe* **17**, 72-84, doi:10.1016/j.chom.2014.11.010 (2015).

15 Hooper, L. V., Littman, D. R. & Macpherson, A. J. Interactions between the microbiota and the immune system. *Science* **336**, 1268-1273, doi:10.1126/science.1223490 (2012).

16 Kau, A. L., Ahern, P. P., Griffin, N. W., Goodman, A. L. & Gordon, J. I. Human nutrition, the gut microbiome and the immune system. *Nature* **474**, 327-336, doi:10.1038/nature10213 (2011).

17 Komaroff, A. L. The Microbiome and Risk for Obesity and Diabetes. *JAMA* **317**, 355-356, doi:10.1001/jama.2016.20099 (2017).

18 Kostic, A. D. *et al.* Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe* **14**, 207-215, doi:10.1016/j.chom.2013.07.007 (2013).

19 Zegarra-Ruiz, D. F. *et al.* A Diet-Sensitive Commensal Lactobacillus Strain Mediates TLR7-Dependent Systemic Autoimmunity. *Cell Host Microbe* **25**, 113-127 e116, doi:10.1016/j.chom.2018.11.009 (2019).

20 Marino, E. *et al.* Gut microbial metabolites limit the frequency of autoimmune T cells and protect against type 1 diabetes. *Nat Immunol* **18**, 552-562, doi:10.1038/ni.3713 (2017).

21 Thorburn, A. N. *et al.* Evidence that asthma is a developmental origin disease influenced by maternal diet and bacterial metabolites. *Nat Commun* **6**, 7320, doi:10.1038/ncomms8320 (2015).

22 Cenit, M. C., Sanz, Y. & Codoner-Franch, P. Influence of gut microbiota on neuropsychiatric disorders. *World J Gastroenterol* **23**, 5486-5498, doi:10.3748/wjg.v23.i30.5486 (2017).

23 Vogt, N. M. *et al.* Gut microbiome alterations in Alzheimer's disease. *Sci Rep* **7**, 13537, doi:10.1038/s41598-017-13601-y (2017).

24 Bello, M. G. D., Knight, R., Gilbert, J. A. & Blaser, M. J. Preserving microbial diversity. *Science* **362**, 33-34, doi:10.1126/science.aau8816 (2018).

25 Bokulich, N. A. *et al.* Antibiotics, birth mode, and diet shape microbiome maturation during early life. *Sci Transl Med* **8**, 343ra382, doi:10.1126/scitranslmed.aad7121 (2016).

26 Sanguinetti, E. *et al.* Microbiota signatures relating to reduced memory and exploratory behaviour in the offspring of overweight mothers in a murine model. *Sci Rep* **9**, 12609, doi:10.1038/s41598-019-48090-8 (2019).

27 De Filippo, C. *et al.* Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. *Proc Natl Acad Sci U S A* **107**, 14691-14696, doi:10.1073/pnas.1005963107 (2010).

28 Singh, R. K. *et al.* Influence of diet on the gut microbiome and implications for human health. *J Transl Med* **15**, 73, doi:10.1186/s12967-017-1175-y (2017).

29 Koh, A., De Vadder, F., Kovatcheva-Datchary, P. & Backhed, F. From Dietary Fiber to Host Physiology: Short-Chain Fatty Acids as Key Bacterial Metabolites. *Cell* **165**, 1332-1345, doi:10.1016/j.cell.2016.05.041 (2016).

30 Thorburn, A. N., Macia, L. & Mackay, C. R. Diet, metabolites, and "western-lifestyle" inflammatory diseases. *Immunity* **40**, 833-842, doi:10.1016/j.immuni.2014.05.014 (2014).

31 Olson, C. A. *et al.* The Gut Microbiota Mediates the Anti-Seizure Effects of the Ketogenic Diet. *Cell* **173**, 1728-1741 e1713, doi:10.1016/j.cell.2018.04.027 (2018).

32 David, L. A. *et al.* Diet rapidly and reproducibly alters the human gut microbiome. *Nature* **505**, 559-563, doi:10.1038/nature12820 (2014).

33 Kelly, C. J. *et al.* Crosstalk between Microbiota-Derived Short-Chain Fatty Acids and Intestinal Epithelial HIF Augments Tissue Barrier Function. *Cell Host Microbe* **17**, 662-671, doi:10.1016/j.chom.2015.03.005 (2015).

34 Albenberg, L. *et al.* Correlation between intraluminal oxygen gradient and radial partitioning of intestinal microbiota. *Gastroenterology* **147**, 1055-1063 e1058, doi:10.1053/j.gastro.2014.07.020 (2014).

35 Byndloss, M. X. *et al.* Microbiota-activated PPAR-gamma signaling inhibits dysbiotic Enterobacteriaceae expansion. *Science* **357**, 570-575, doi:10.1126/science.aam9949 (2017).

36 Garrett, W. S. *et al.* Enterobacteriaceae act in concert with the gut microbiota to induce spontaneous and maternally transmitted colitis. *Cell Host Microbe* **8**, 292-300, doi:10.1016/j.chom.2010.08.004 (2010).

37 Rivera-Chavez, F., Lopez, C. A. & Baumler, A. J. Oxygen as a driver of gut dysbiosis. *Free Radic Biol Med* **105**, 93-101, doi:10.1016/j.freeradbiomed.2016.09.022 (2017).

38 Serino, M. Molecular Paths Linking Metabolic Diseases, Gut Microbiota Dysbiosis and Enterobacteria Infections. *J Mol Biol* **430**, 581-590, doi:10.1016/j.jmb.2018.01.010 (2018).

39 Karl, J. P. *et al.* Associations between the gut microbiota and host responses to high altitude. *Am J Physiol Gastrointest Liver Physiol* **315**, G1003-G1015, doi:10.1152/ajpgi.00253.2018 (2018).

40 Desbonnet, L., Clarke, G., Shanahan, F., Dinan, T. G. & Cryan, J. F. Microbiota is essential for social development in the mouse. *Mol Psychiatry* **19**, 146-148, doi:10.1038/mp.2013.65 (2014).

41 Diaz Heijtz, R. *et al.* Normal gut microbiota modulates brain development and behavior. *Proc Natl Acad Sci U S A* **108**, 3047-3052, doi:10.1073/pnas.1010529108 (2011).

42 Gareau, M. G. *et al.* Bacterial infection causes stress-induced memory dysfunction in mice. *Gut* **60**, 307-317, doi:10.1136/gut.2009.202515 (2011).

43 Sampson, T. R. *et al.* Gut Microbiota Regulate Motor Deficits and Neuroinflammation in a Model of Parkinson's Disease. *Cell* **167**, 1469-1480 e1412, doi:10.1016/j.cell.2016.11.018 (2016).

44 Cognitive Impairment: A Call for Action, Now! , (Centers for Disease Control and Prevention, 2011).

45 Arciniegas, D. B., Held, K. & Wagner, P. Cognitive Impairment Following Traumatic Brain Injury. *Curr Treat Options Neurol* **4**, 43-57, doi:10.1007/s11940-002-0004-6 (2002).

46 Vuong, H. E., Yano, J. M., Fung, T. C. & Hsiao, E. Y. The Microbiome and Host Behavior. *Annu Rev Neurosci* **40**, 21-49, doi:10.1146/annurev-neuro-072116-031347 (2017).

47 Lee, H. J., Lee, K. E., Kim, J. K. & Kim, D. H. Suppression of gut dysbiosis by Bifidobacterium longum alleviates cognitive decline in 5XFAD transgenic and aged mice. *Sci Rep* **9**, 11814, doi:10.1038/s41598-019-48342-7 (2019).

48 Zhao, M. *et al.* Ketogenic diet improves the spatial memory impairment caused by exposure to hypobaric hypoxia through increased acetylation of histones in rats. *PLoS One* **12**, e0174477, doi:10.1371/journal.pone.0174477 (2017).

49 Sovran, B. *et al.* Age-associated Impairment of the Mucus Barrier Function is Associated with Profound Changes in Microbiota and Immunity. *Sci Rep* **9**, 1437, doi:10.1038/s41598-018-35228-3 (2019).

50 Ticinesi, A., Nouvenne, A., Tana, C., Prati, B. & Meschi, T. Gut Microbiota and Microbiota-Related Metabolites as Possible Biomarkers of Cognitive Aging. *Adv Exp Med Biol* **1178**, 129-154, doi:10.1007/978-3-030-25650-0_8 (2019).

51 Jarrard, L. E. On the role of the hippocampus in learning and memory in the rat. *Behav Neural Biol* **60**, 9-26, doi:10.1016/0163-1047(93)90664-4 (1993).

52 Stuchlik, A. Dynamic learning and memory, synaptic plasticity and neurogenesis: an update. *Front Behav Neurosci* **8**, 106, doi:10.3389/fnbeh.2014.00106 (2014).

53 Suarez, A. N. *et al.* Gut vagal sensory signaling regulates hippocampus function through multi-order pathways. *Nat Commun* **9**, 2181, doi:10.1038/s41467-018-04639-1 (2018).

54 Fulling, C., Dinan, T. G. & Cryan, J. F. Gut Microbe to Brain Signaling: What Happens in Vagus. *Neuron* **101**, 998-1002, doi:10.1016/j.neuron.2019.02.008 (2019).

55 Chu, C. *et al.* The microbiota regulate neuronal function and fear extinction learning. *Nature* **574**, 543-548, doi:10.1038/s41586-019-1644-y (2019).

56 Rapp, P. R. & Gallagher, M. Preserved neuron number in the hippocampus of aged rats with spatial learning deficits. *Proc Natl Acad Sci U S A* **93**, 9926-9930, doi:10.1073/pnas.93.18.9926 (1996).

57 Burke, S. N. & Barnes, C. A. Neural plasticity in the ageing brain. *Nat Rev Neurosci* **7**, 30-40, doi:10.1038/nrn1809 (2006).

58 De Felice, F. G., Lourenco, M. V. & Ferreira, S. T. How does brain insulin resistance develop in Alzheimer's disease? *Alzheimers Dement* **10**, S26-32, doi:10.1016/j.jalz.2013.12.004 (2014).

59 Underwood, E. L. & Thompson, L. T. High-fat diet impairs spatial memory and hippocampal intrinsic excitability and sex-dependently alters circulating insulin and hippocampal insulin sensitivity. *Biol Sex Differ* **7**, 9, doi:10.1186/s13293-016-0060-3 (2016).

60 Zanini, P. *et al.* Diet-induced obesity alters memory consolidation in female rats. *Physiol Behav* **180**, 91-97, doi:10.1016/j.physbeh.2017.08.011 (2017).

61 Hernandez, A. R. *et al.* Age and Ketogenic Diet Have Dissociable Effects on Synapse-Related Gene Expression Between Hippocampal Subregions. *Front Aging Neurosci* **11**, 239, doi:10.3389/fnagi.2019.00239 (2019).

62 Reikvam, D. H. *et al.* Depletion of murine intestinal microbiota: effects on gut mucosa and epithelial gene expression. *PLoS One* **6**, e17996, doi:10.1371/journal.pone.0017996 (2011).

63 Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114-2120, doi:10.1093/bioinformatics/btu170 (2014).

64 Kim, D., Paggi, J. M., Park, C., Bennett, C. & Salzberg, S. L. Graph-based genome alignment and genotyping with HISAT2 and HISAT-genotype. *Nat Biotechnol* **37**, 907-915, doi:10.1038/s41587-019-0201-4 (2019).

65 Anders, S., Pyl, P. T. & Huber, W. HTSeq--a Python framework to work with high-throughput sequencing data. *Bioinformatics* **31**, 166-169, doi:10.1093/bioinformatics/btu638 (2015).

66 Love, M. I., Huber, W. & Anders, S. Moderated estimation of fold change and dispersion for RNA-seq data with DESeq2. *Genome Biol* **15**, 550, doi:10.1186/s13059-014-0550-8 (2014).

67 McLeod, F., Marzo, A., Podpolny, M., Galli, S. & Salinas, P. Evaluation of Synapse Density in Hippocampal Rodent Brain Slices. *J Vis Exp*, doi:10.3791/56153 (2017).

68 Hota, K. B., Hota, S. K., Srivastava, R. B. & Singh, S. B. Neuroglobin regulates hypoxic response of neuronal cells through Hif-1alpha- and Nrf2-mediated mechanism. *J Cereb Blood Flow Metab* **32**, 1046-1060, doi:10.1038/jcbfm.2012.21 (2012).

69 Wenzel, H. J., Cole, T. B., Born, D. E., Schwartzkroin, P. A. & Palmiter, R. D. Ultrastructural localization of zinc transporter-3 (ZnT-3) to synaptic vesicle membranes within mossy fiber boutons in the hippocampus of mouse and monkey. *Proc Natl Acad Sci U S A* **94**, 12676-12681, doi:10.1073/pnas.94.23.12676 (1997).

70 Jonas, P. & Lisman, J. Structure, function, and plasticity of hippocampal dentate gyrus microcircuits. *Front Neural Circuits* **8**, 107, doi:10.3389/fncir.2014.00107 (2014).

71 Daumas, S., Halley, H., Frances, B. & Lassalle, J. M. Encoding, consolidation, and retrieval of contextual memory: differential involvement of dorsal CA3 and CA1 hippocampal subregions. *Learn Mem* **12**, 375-382, doi:10.1101/lm.81905 (2005).

72 Rolls, E. T. A computational theory of episodic memory formation in the hippocampus. *Behav Brain Res* **215**, 180-196, doi:10.1016/j.bbr.2010.03.027 (2010).

73 Squire, L. R. & Wixted, J. T. The cognitive neuroscience of human memory since H.M. *Annu Rev Neurosci* **34**, 259-288, doi:10.1146/annurev-neuro-061010-113720 (2011).

74 Jin, J. L. *et al.* CART treatment improves memory and synaptic structure in APP/PS1 mice. *Sci Rep* **5**, 10224, doi:10.1038/srep10224 (2015).

75 Monzon, M. E. *et al.* Melanin-concentrating hormone (MCH) modifies memory retention in rats. *Peptides* **20**, 1517-1519, doi:10.1016/s0196-9781(99)00164-3 (1999).

76 He, Y. *et al.* Targeted deletion of a single Sca8 ataxia locus allele in mice causes abnormal gait, progressive loss of motor coordination, and Purkinje cell dendritic deficits. *J Neurosci* **26**, 9975-9982, doi:10.1523/JNEUROSCI.2595-06.2006 (2006).

77 Schurmans, S. *et al.* Impaired long-term potentiation induction in dentate gyrus of calretinin-deficient mice. *Proc Natl Acad Sci U S A* **94**, 10415-10420, doi:10.1073/pnas.94.19.10415 (1997).

78 Wojcik, S. M. *et al.* Genetic markers of a Munc13 protein family member, BAIAP3, are gender specifically associated with anxiety and benzodiazepine abuse in mice and humans. *Mol Med* **19**, 135-148, doi:10.2119/molmed.2013.00033 (2013).

79 Marrocco, J. *et al.* Early Life Stress Restricts Translational Reactivity in CA3 Neurons Associated With Altered Stress Responses in Adulthood. *Front Behav Neurosci* **13**, 157, doi:10.3389/fnbeh.2019.00157 (2019).

80 Rosenzweig, N. *et al.* PD-1/PD-L1 checkpoint blockade harnesses monocyte-derived macrophages to combat cognitive impairment in a tauopathy mouse model. *Nat Commun* **10**, 465, doi:10.1038/s41467-019-08352-5 (2019).

81 Ianov, L. *et al.* Hippocampal Transcriptomic Profiles: Subfield Vulnerability to Age and Cognitive Impairment. *Front Aging Neurosci* **9**, 383, doi:10.3389/fnagi.2017.00383 (2017).

82 Mehta, A., Prabhakar, M., Kumar, P., Deshmukh, R. & Sharma, P. L. Excitotoxicity: bridge to various triggers in neurodegenerative disorders. *Eur J Pharmacol* **698**, 6-18, doi:10.1016/j.ejphar.2012.10.032 (2013).

83 Moreno-Jimenez, E. P. *et al.* Adult hippocampal neurogenesis is abundant in neurologically healthy subjects and drops sharply in patients with Alzheimer's disease. *Nat Med* **25**, 554-560, doi:10.1038/s41591-019-0375-9 (2019).