

Using Neuroimaging to Study the Effects of Probiotics on the Human Brain

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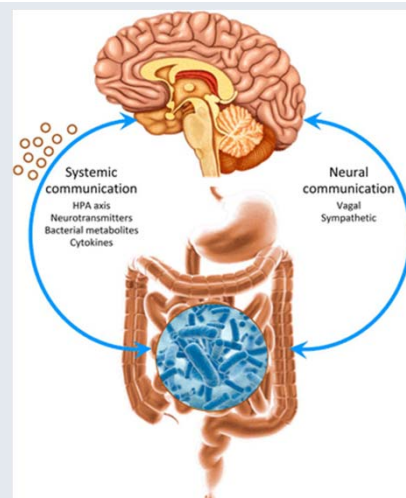
Outline

- Review of literature demonstrating effect gut microbiota can have on the neurological diseases and neuropsychological disorders
 - Possible mechanisms for how microbiome can affect the central nervous system (CNS)
 - Beyond mood and satiety strong evidence that the microbiome can influence anxiety, memory, and pain processing
- Describe how neuroimaging can be used to noninvasively measure changes in the CNS via probiotics and prebiotics
 - Function: fMRI
 - Morphometry: gray-matter volume, lesion measurements, white matter integrity
 - Metabolite concentrations: MR Spectroscopy (MRS)
- Examples
- Summary

Mechanisms by which Probiotics Can Affect the CNS

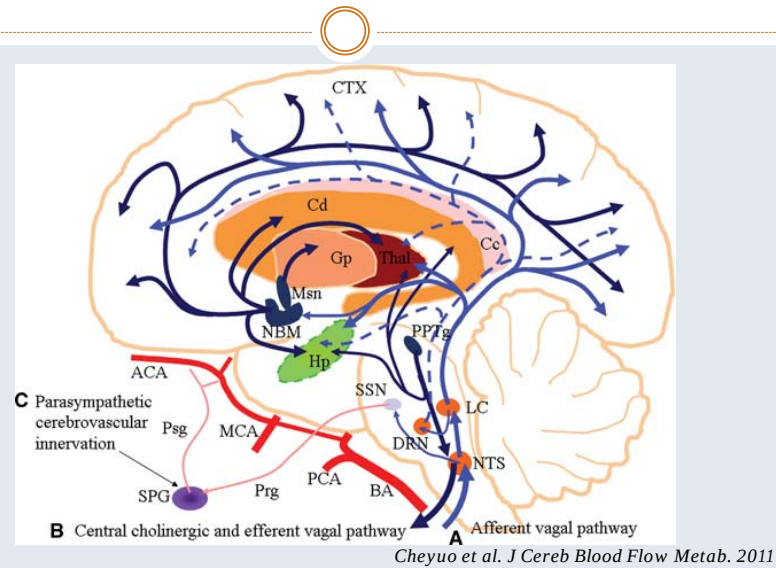
Interaction Between Gut Microbiota and CNS

- Growing body of literature in animal models has provided evidence of bidirectional signaling between the brain and the gut microbiota
- Three potential mechanisms of signaling between the two:
 - Vagus nerve – direct connection
 - Immune cells – circulatory
 - Metabolites produced by microbes in the gut – needs more work

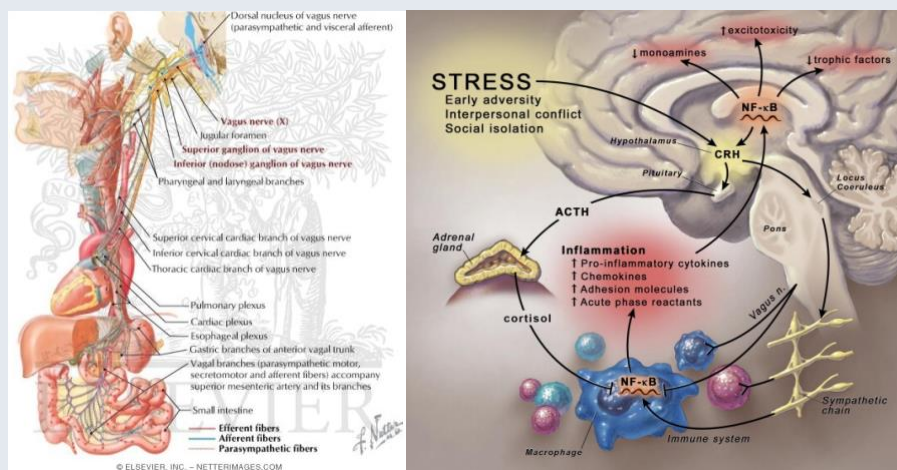


Mayer et al. J Neurosci. 2014

Vagus Nerve – Cortical Pathways



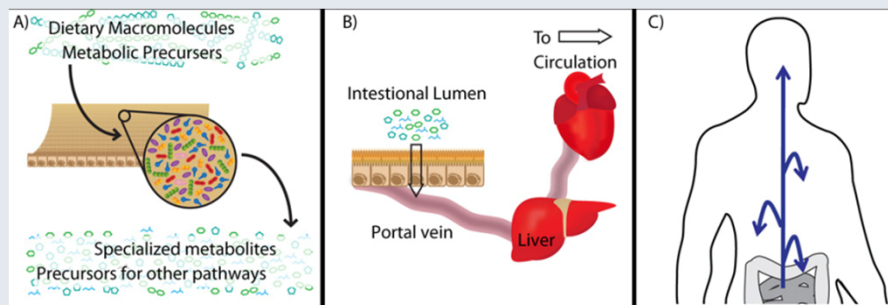
Vagus Nerve - Organs and Stress



Source: <http://drsircus.com/medicine/vagus-nerve-inflammation-heart-rate-variability>

From the Gut to the Brain

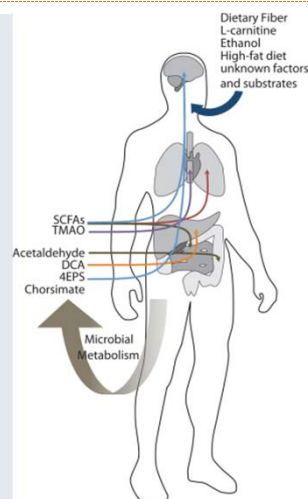
- Metabolites produced in the gut must pass through mucosal epithelium, which regulates passage of essential nutrients into circulation
- Liver filters any particulate matter and bacteria
- Soluble metabolites and nutrients are absorbed and enter the circulation
- Insoluble, fat-based metabolites are most commonly absorbed into the lymphatic system and into the bloodstream through the thoracic duct



Sharon et al. Cell Metab. 2014

Possible Metabolites Related to Probiotics

- Short chain fatty acids (SCFAs) including propionate, butyrate, and acetate
 - Acetate - acetate crosses the blood-brain barrier, where it is taken up and activates hypothalamic neurons
- maternal immune activation (MIA) mouse model for autism spectrum disorders (ASD)... administration of a probiotic, *Bacteroides fragilis* NTCC 9343, corrected some of the behavioral deficits and restored levels of some serum metabolites. One of these metabolites, namely 4-ethylphenylsulfate (4-EPS), was sufficient to induce anxiety-like behaviors in mice

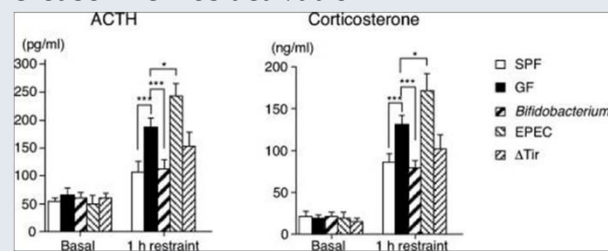


Sharon et al. Cell Metab. 2014

Review of Affect of Probiotics on the CNS

Probiotic Affect on Stress Response

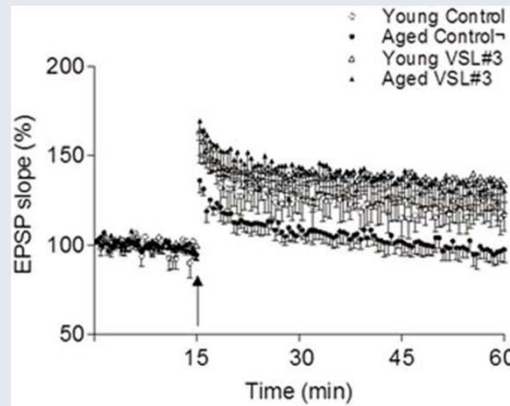
- *Bifidobacterium infantis* reverses heightened hypothalamic–pituitary–adrenal (HPA) stress reactivity in germ-free (GF) mice
- Hypothesized that either stimulated immune cells within the gut to release cytokines (IL-1) or vagus nerve given rapid increase in c-Fos activation



Sudo et al. J Physiol. 2004

Probiotic Reversal of Age-related Memory Deficits

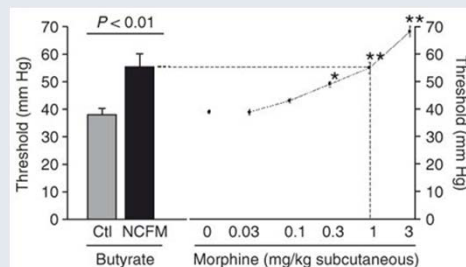
- VSL#3 mixture of eight different probiotics
 - Reversed age-related deficits in long-term potentiation (the basis of memory)
 - Increased expression of brain-derived neurotrophic factor (BDNF), which induces neurogenesis



Distrutti et al. PLoS One. 2014

Probiotic Modulation of Pain

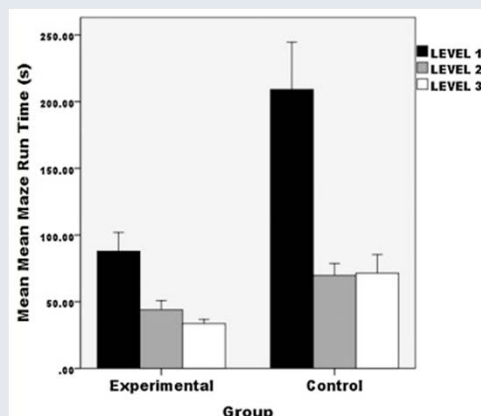
- Colorectal distension used to induce pain
- *L. acidophilus* NCFM reduced visceral pain through expression of μ -opioid and cannabinoid receptors in intestinal epithelial cells
- Increased pain threshold by 20%
- Probiotics may exert local control of the transmission of nociceptive information to the intestinal nervous system by inducing the expression of receptors on epithelial cells



Rousseaux et al. PLoS One. 2014

Microbe Modulation of Anxiety

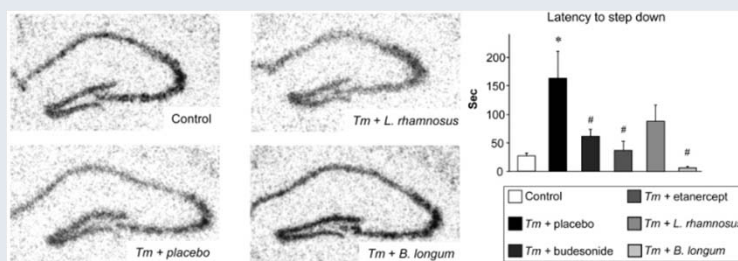
- *Mycobacterium vaccae* cannot colonize the anaerobic GI tract of animals but is thought of as a transient commensal
- Reduced anxiety and improved performance on a complex maze task
- *M. vaccae* may stimulate serotonin production through immunological mechanisms



Matthews and Jenks Behav Processes 2013

Probiotic Modification of BDNF and Anxiety

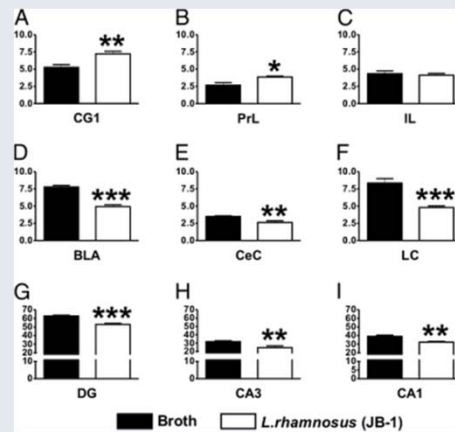
- AKR mice (n = 95) were infected with T muris which caused mild to moderate colonic inflammation and anxiety-like behavior
- Probiotic *Bifidobacterium longum* normalized behavior and increased hippocampal BDNF mRNA expression
- Notably Tm mice were still infected but their behavior and brain were changed by *B. longum*!



Bercik et al. Gastroenterology 2010

Probiotic Affect on Neurotransmitters

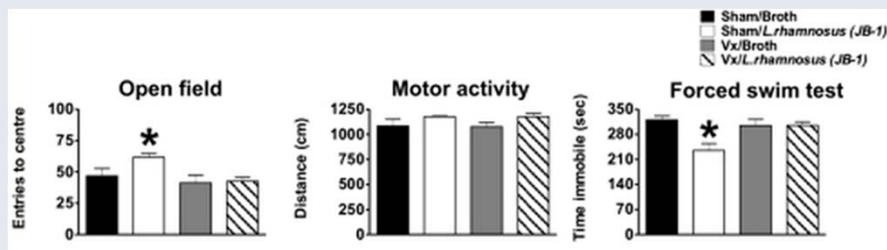
- *L. rhamnosus* (JB-1) differentially affected GABA_{B1b} mRNA expression
- Increased in cortical regions (cingulate and prelimbic)
- Reduced in the hippocampus, amygdala, and locus coeruleus



Bravo et al. PNAS 2011

GABA Modulation via Vagus Nerve

- *L. rhamnosus* reduced stress-induced corticosterone and anxiety- and depression-related behavior.
- Affect disappeared in vagotomized mice thus pinpointing communication between the gut and the brain to the vagus nerve



Bravo et al. PNAS 2011

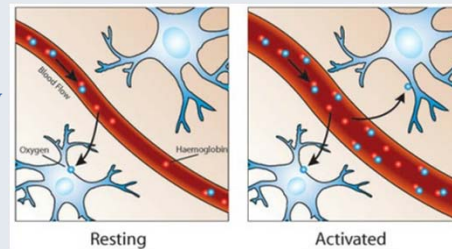
Basic Neuroimaging Techniques

Moving from the Mouse to the Human

- Preclinical studies have provided ample evidence for the effect different probiotics on stress response, pain tolerance, memory, neurotrophin growth factors, and neurotransmitters
- ***Is it possible to non-invasively measure some or all of these changes in the human brain?***
 - *Different MRI-based imaging techniques can inform us about some of these effects*

fMRI – Neuronal Activity

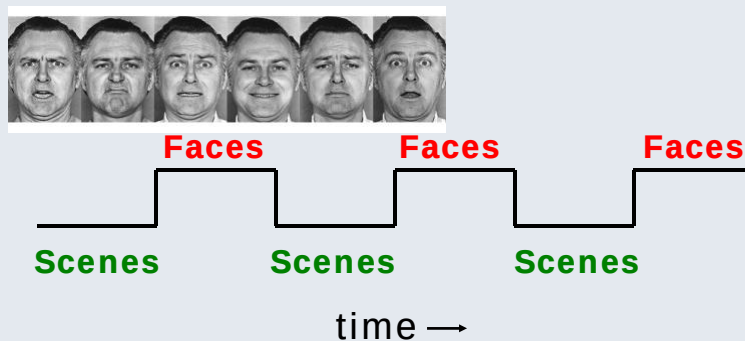
- Indirect measure of blood flow and by inference neuronal activity
 - Areas that are more active require more blood to supply the components of energy metabolism (oxygen and glucose) to neurons
 - fMRI relies on the magnetic properties of deoxygenated hemoglobin to change the MRI signal



Source: <http://www.fmrib.ox.ac.uk/research/education>

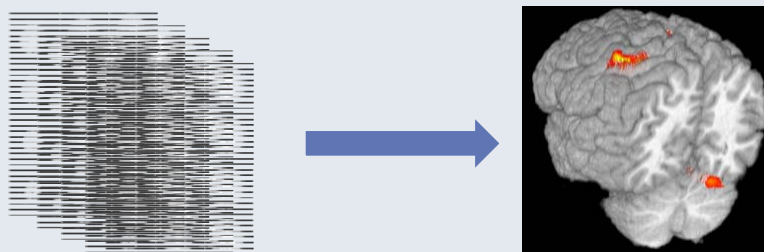
The fMRI Experiment

- Every fMRI experiment uses a “task” in which the subject is instructed to perform specific actions at prescribed points in time

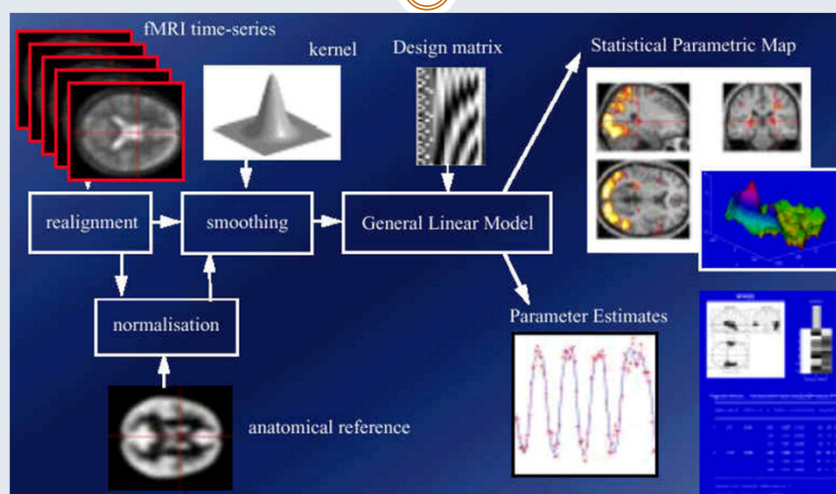


fMRI Preprocessing

- After we collect the fMRI data *a lot of preprocessing* is performed before we can do statistical tests and make pictures of activity

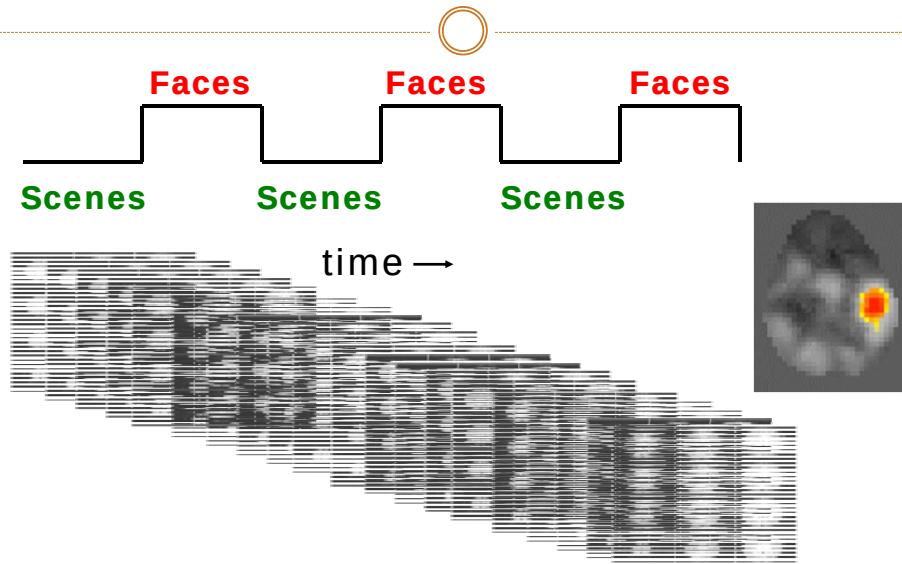


Basic Processing Chain

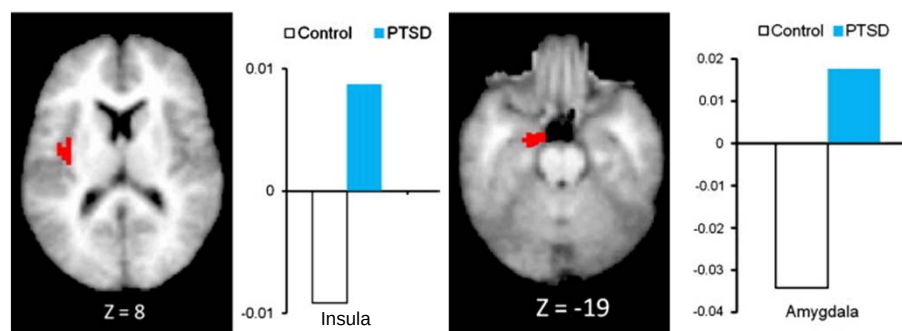


SPM Course, <http://www.fil.ion.ucl.ac.uk/spm/course>

Statistics Used to Find Voxels Whose Signal Changes Match the Experiment



Emotional Face Processing PTSD



Bruce et al Neuroimage Clin 2013

Assessing Differences in Brain Structure

- Lesion measurements
 - Manual counting of number of lesions
- Manual measures of volume
 - Manual tracing of certain brain structures
- Voxel-Based Morphometry (VBM)
 - Automated method for measuring differences in gray matter volume on a voxel-by-voxel basis
- Diffusion Tensor Imaging (DTI)
 - Measure of white matter integrity

Voxel-Based Morphometry (VBM)

- Voxel-Based Morphometry – identifies regions with differences in gray-matter, white-matter, or Cerebrospinal fluid (CSF) concentration
- Relies on segmentation algorithms to classify each voxel in an MRI by the type of tissue

Processing Steps:

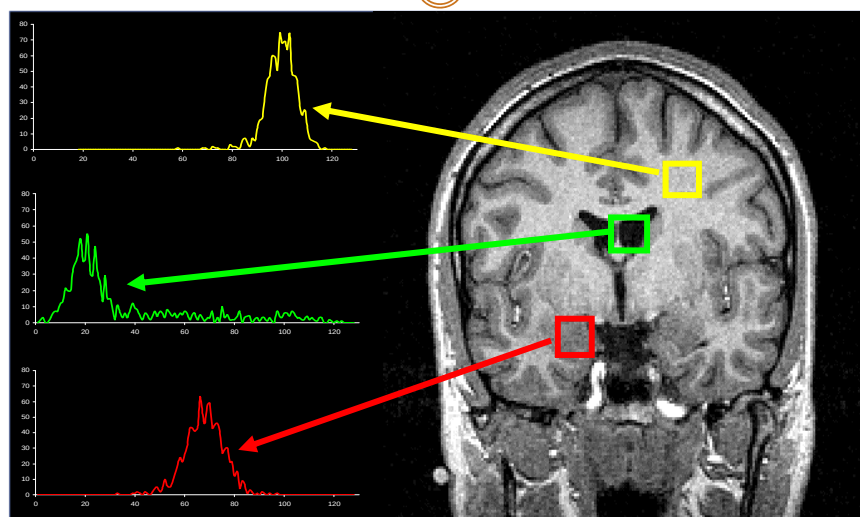
- Spatially normalize – warp everyone's brain into a common atlas space
- Segment each brain into gray matter, white matter, and CSF
- Statistically compare on a voxel-by-voxel basis gray matter probabilities or concentration between subject groups

Tissue Segmentation

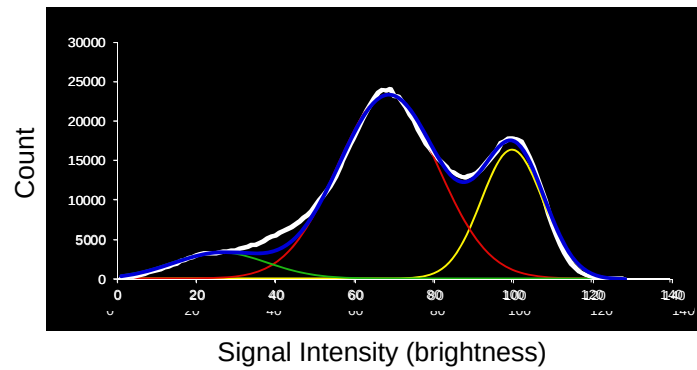
- Classify voxels in MR images of the brain into gray matter, white matter, and CSF
- Use intensities and classification of neighboring voxels to perform classification



Tissue Segmentation

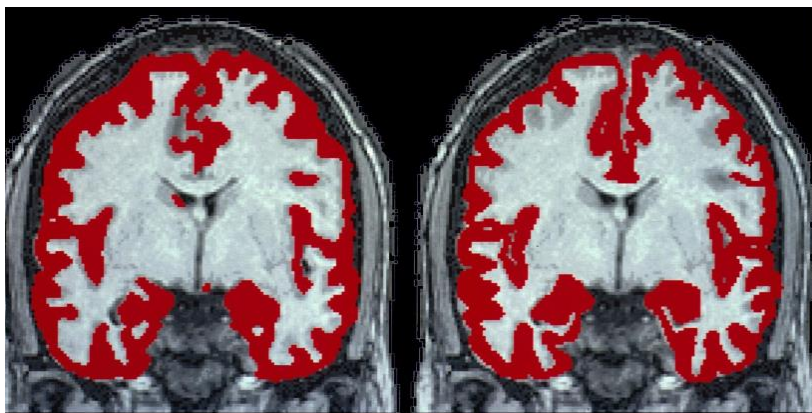


Tissue Segmentation



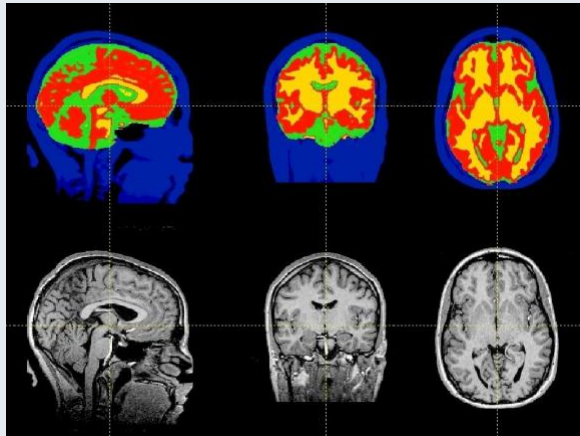
Use histograms to determine range of intensities for each tissue class

Tissue Segmentation



3D Segmentation

- Percentage of each tissue in a voxel can be turned into concentration to give gray-matter, white-matter, and CSF volume
- Populations and/or treatment conditions can be compared

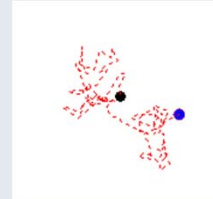


Diffusion Tensor Imaging (DTI)

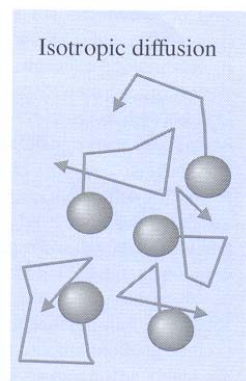
- MR imaging technique in which contrast is based on differences in the diffusion of water molecules
- DTI used to identify and generate maps of white matter fibers

Diffusion Tensor Imaging

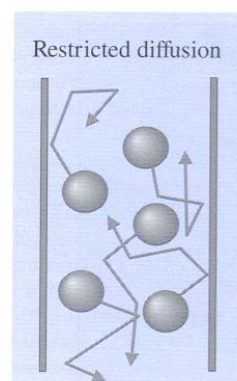
- Diffusion-tensor MRI relates image intensities to the relative mobility of water molecules in tissue and the direction of the motion
- Motion of a water molecules is a random walk (Brownian motion)
- Areas with relatively high mean diffusion will appear dark on the Diffusion weighted MRI images



Types of Molecular Motion

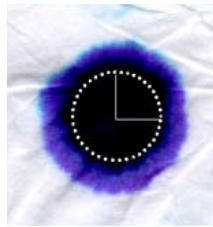


Diffusion in an open pool of fluid (Ventricles)

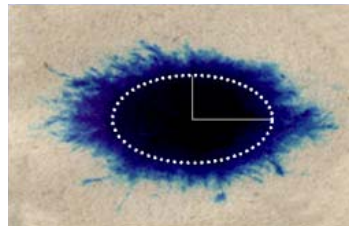


Diffusion in an axon

Types of Isotropy



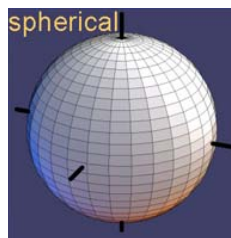
Kleenex



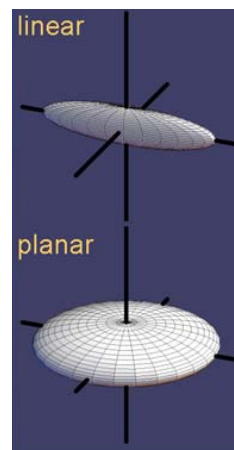
Newspaper



Types of 3D Isotropy



Isotropic



Anisotropic

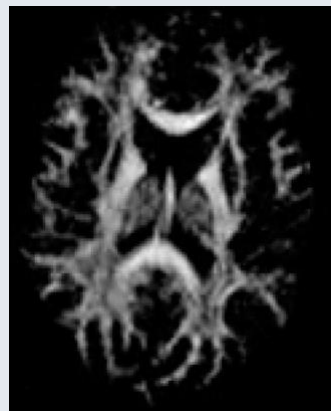
DTI Calculation

- Diffusion properties described with a 3 X 3 symmetric tensor matrix
- Eigenvalues of the diffusion tensor are the diffusion coefficients (λ_x , λ_y , and λ_z) in the three principal directions of diffusivity
- The eigenvector corresponding to the largest eigenvalue is the main direction of diffusion
- Maps are constructed from various anisotropy indices calculated from the eigenvalues

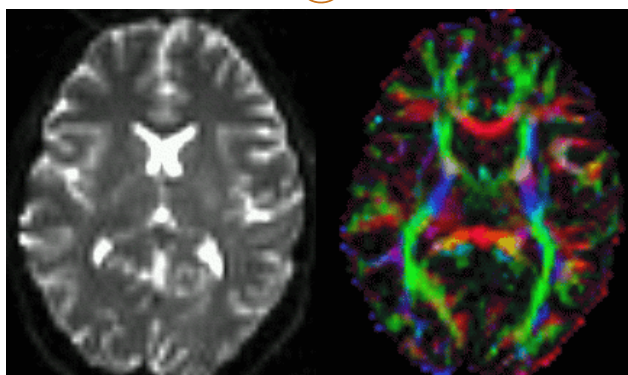
Fractional Anisotropy (FA) Index

- Measure of degree of anisotropy regardless of direction
- Brighter areas correspond to areas with higher degree of diffusion

$$FA = \sqrt{\frac{3(\lambda_x - \lambda_y)^2 + (\lambda_x - \lambda_z)^2 + (\lambda_y - \lambda_z)^2}{2(\lambda_x^2 + \lambda_y^2 + \lambda_z^2)}}$$



Direction of Diffusion

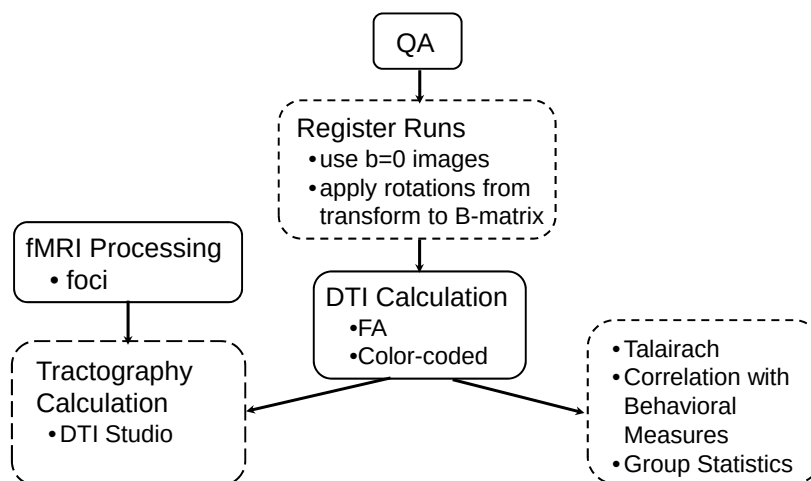


Red = Left-Right

Green = Anterior-Posterior

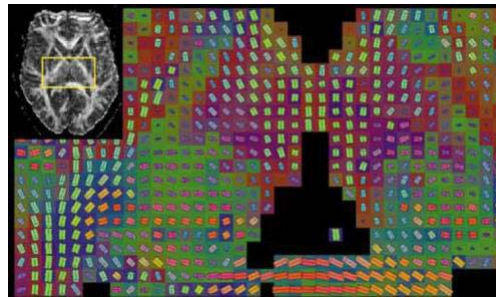
Blue = Superior-Inferior

DTI Data Processing

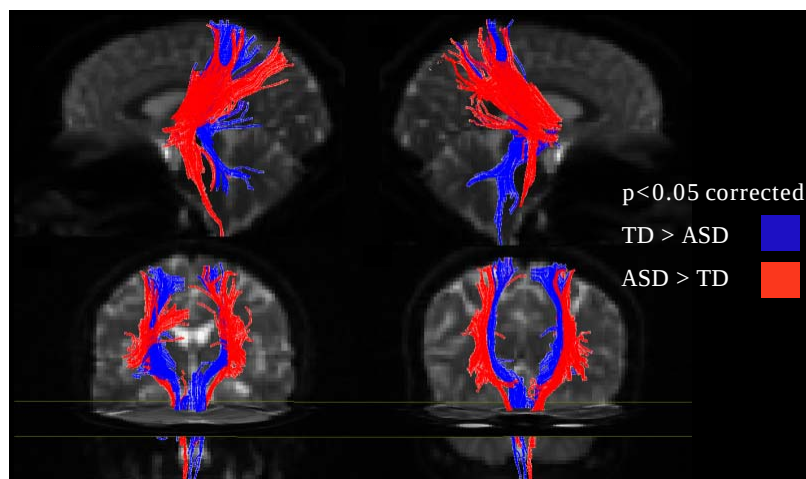


Tractography

- Use primary direction calculated from tensor as input
- Connect neighboring voxels if the tensor in one points towards the other
- Tracts built from a collection of connected voxels



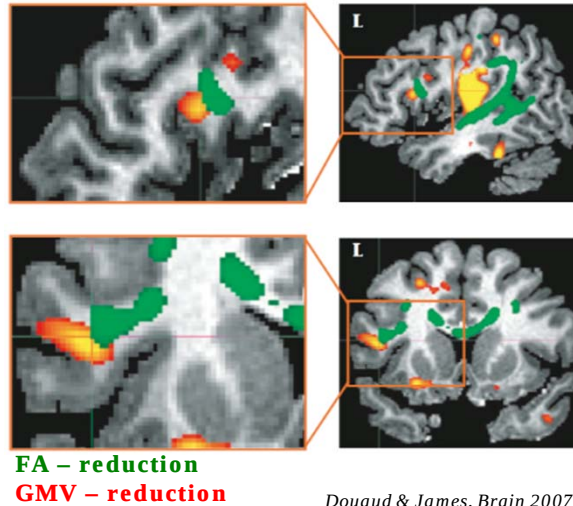
Autistic versus Typical Developing Children



VanMeter et al. in preparation

Combining VBM and DTI

- Adolescent-onset schizophrenia
- Complimentary techniques that examine gray matter volume (GMV) and white matter integrity (FA)

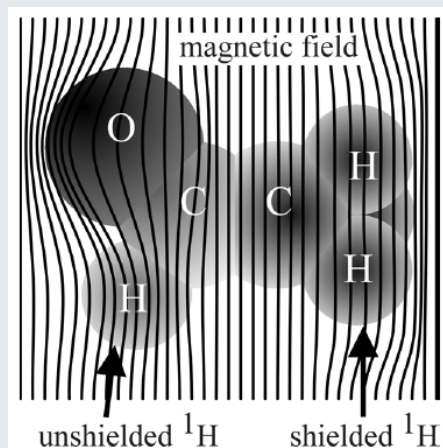


MRI Spectroscopy

- Technique to examine concentrations of various metabolites
- Same technique used in NMR spectroscopy
- Using data collected on phantoms with known concentration allows for quantification concentration
- Major metabolites of interest:

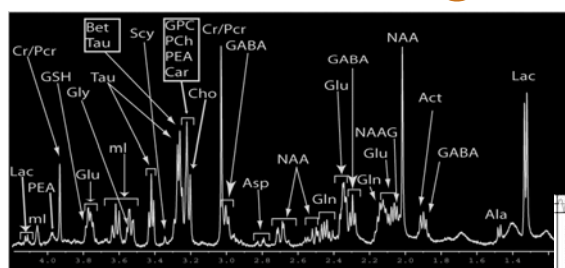
GABA	Lactate	N-acetylaspartate
Glutamate	Choline	Myo-inositol
Glutamine	Creatine	

Shielding and Chemical Shift



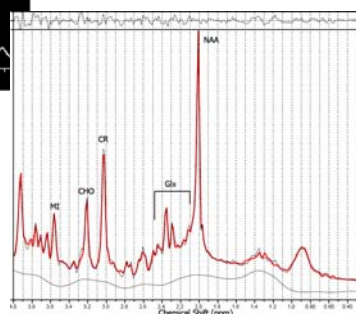
- Human MRI scanners tuned for hydrogen
- Metabolites with hydrogen can be detected by shift in frequency at which the hydrogen protons precess
- Shift results from shielding by other atoms in the molecule

^1H Metabolites



Ex vivo at 11T

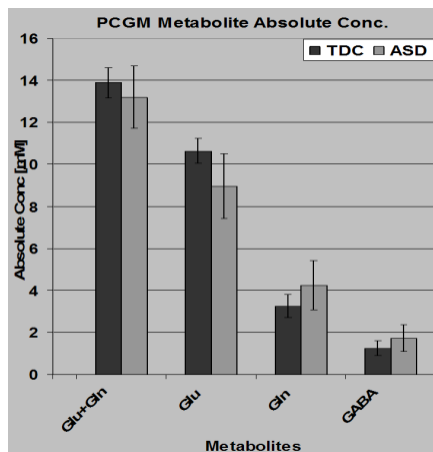
In vivo at 3T



MRS Differences in Autism

- Significant decrease in Glutamate
- Significant increase in Glutamine and GABA
- Recent mapping of autism genetic linkage implicates glutamate related chromosome 11p12-p13 (neurexin 1)*

*Autism Genome Project, Nature Genetics, March 2007

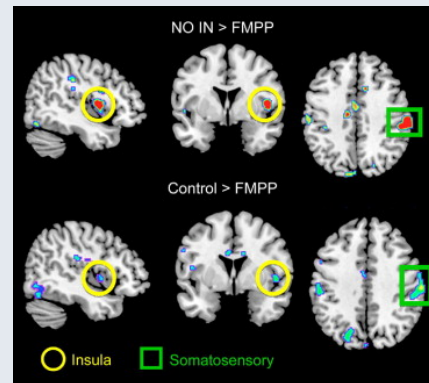


VanMeter et al. in preparation

Examples of Application of Neuroimaging to Examine Affect of Probiotics

fMRI and Probiotics

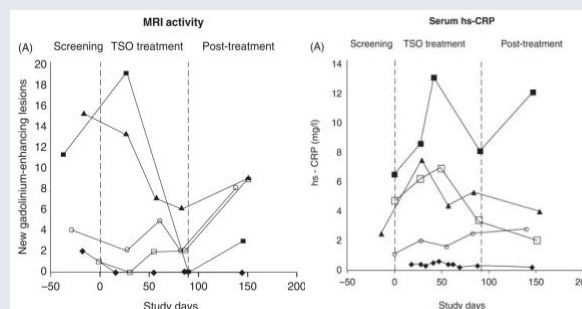
- Subjects were scanned before and after intervention:
 - Fermented milk product with probiotics (n=12, FMPP)
 - A nonfermented milk product (n = 11, Controls)
 - No intervention (n=13, NoIn)



Tillisch et al. Gastroenterology 2013

Formation of New Lesions in Multiple Sclerosis

- Contrast-enhanced MRI used to measure changes in the number of multiple sclerosis following treatment with *Trichuris suis* (TSO)
- Number of new lesions (n-Gd+) fell from 6.6 at baseline to 2.0 at the end of TSO administration
- 2 months after TSO was discontinued number of lesions rose to 5.8



Fleming et al. Mult Scler. 2011

Summary



- Animal models have provided strong evidence that probiotics can have a dramatic affect on the central nervous system
 - stress response
 - pain tolerance
 - memory
 - neurotrophin growth factors
 - neurotransmitters
- More work is needed to identify mechanisms by which these affects are produced
- Neuroimaging techniques provide a range of techniques for measuring changes due to probiotics *in vivo* particularly in human studies

fMRI

VBM, DTI

MRS