From Blood-brain Barrier to Blood-brain Interface: Strategies for Drug Delivery to the Brain

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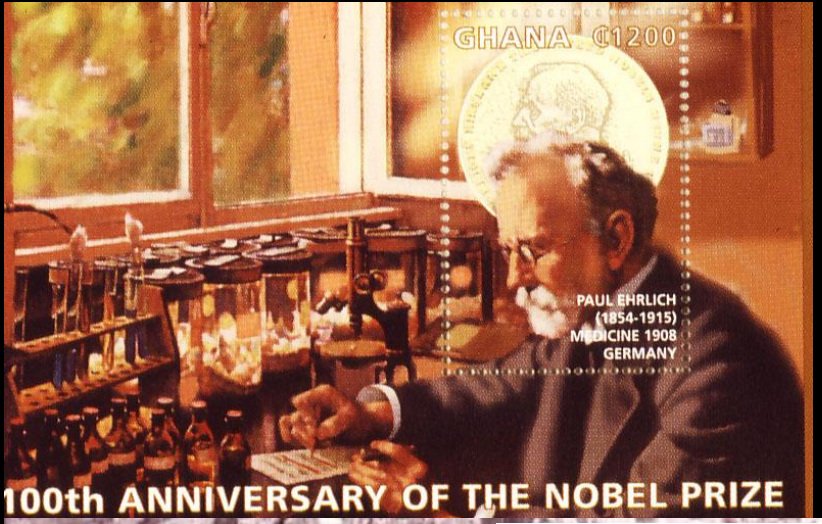
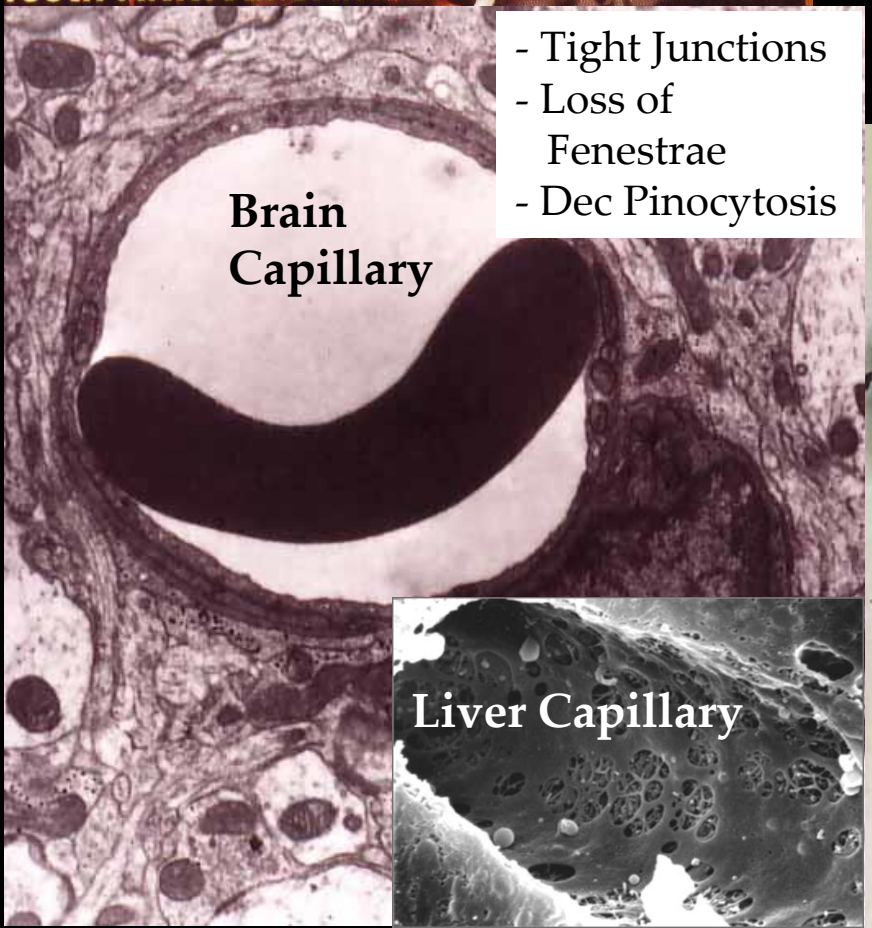


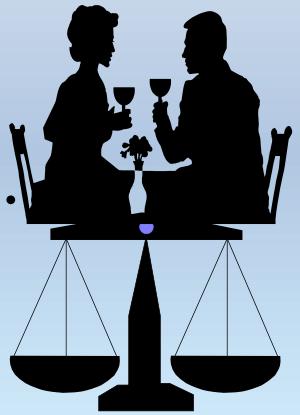


- Tight Junctions
- Loss of Fenestrae
- Dec Pinocytosis

Beyond the Barrier: Roles of the Blood-Brain Barrier

- **Nutrition**

  Glucose, Amino Acids, FFA, Vitamins.



- **Homeostasis**

  Electrolytes, HCO3, p-Glycoprotein...

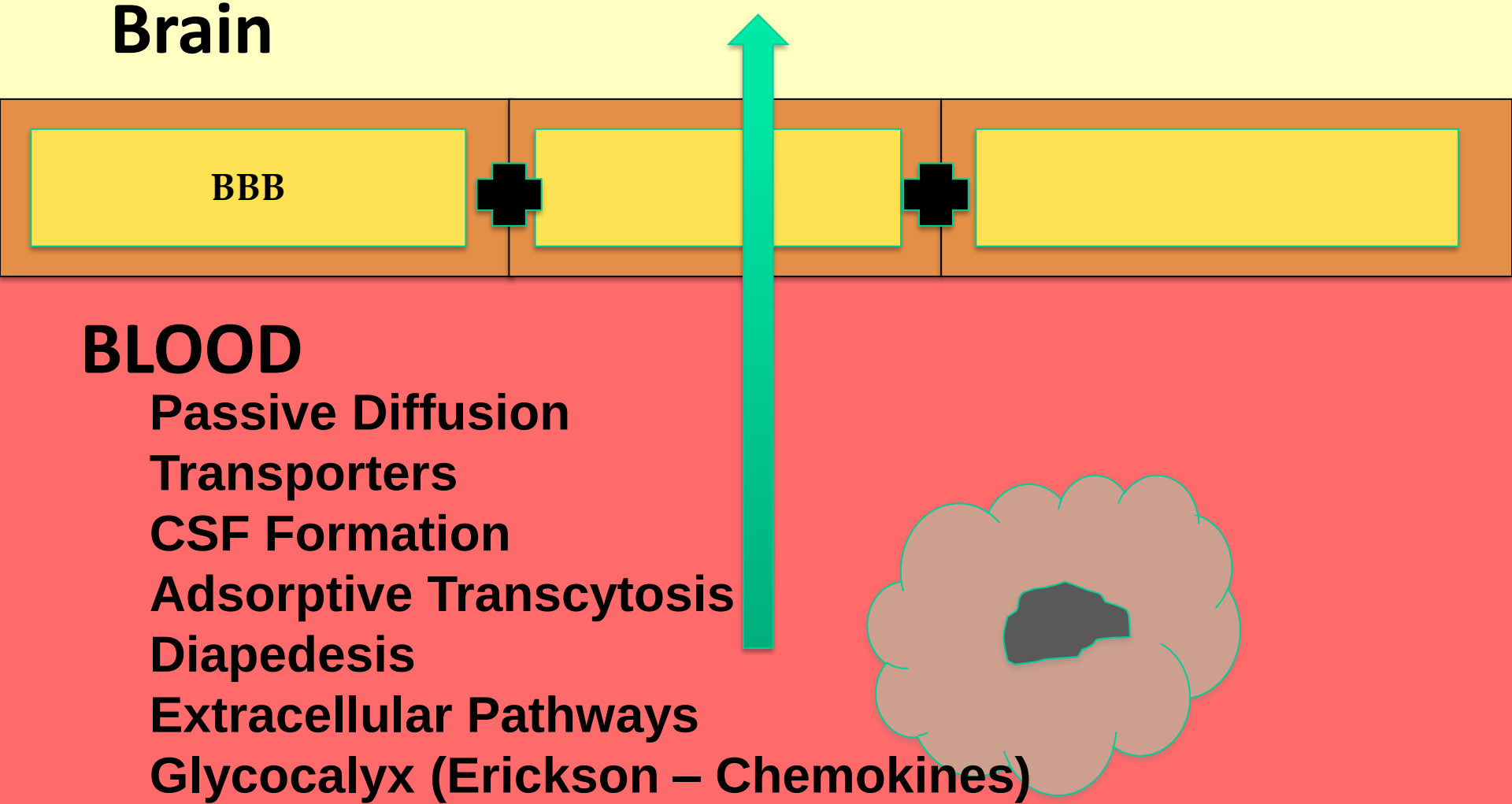
- **Communication**

  Peptides & Regulatory Proteins

  (Leptin, Enkephalins, Cytokines….)

Blood-Brain Interface

How Do Substances X the BBB?



100% Biologics Do Not Cross the BBB

Insulin
IGF-1
Transferrin

Leptin
Ghrelin
Pan Polypep.

Prolactin
GH
LH

TNF-alpha
IL-1 alpha
IL-1 beta
IL-1 ra: Anakinra
IL-6
GM-CSF
BDNF
FGFs
Chemokines

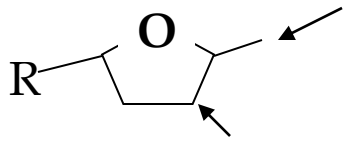
Delta Sleep Inducing Peptide
Vasoactive Intestinal Peptide
PACAP1-39
PACAP1-27
MSH
Secretin
Substance P
B-Endorphin
Abeta Peptide
Alpha-Synuclein
Murine Prion
LHRH
MIF-1
AVP
HMGB-1
S Protein (Covid)
GP120 (HIV-1)

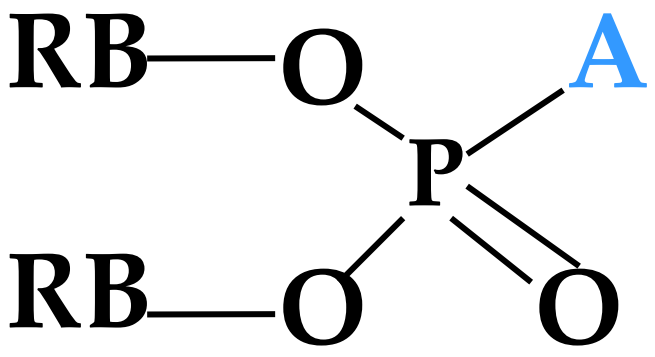
Cyclo His-Pro
Amylin
Orexin-A
Tat
LIF-1
Nesfatin-1
Exendin
Breaker Peptides

Peptide Analogs
Oligophosphoro-thioate Antisenses
Glycoproteins
Apolipoproteins
Triglycerides
Exosomes
Viruses/Bacteria/Parasites

Oligophosphorothioate Antisenses

R = Nucleoside

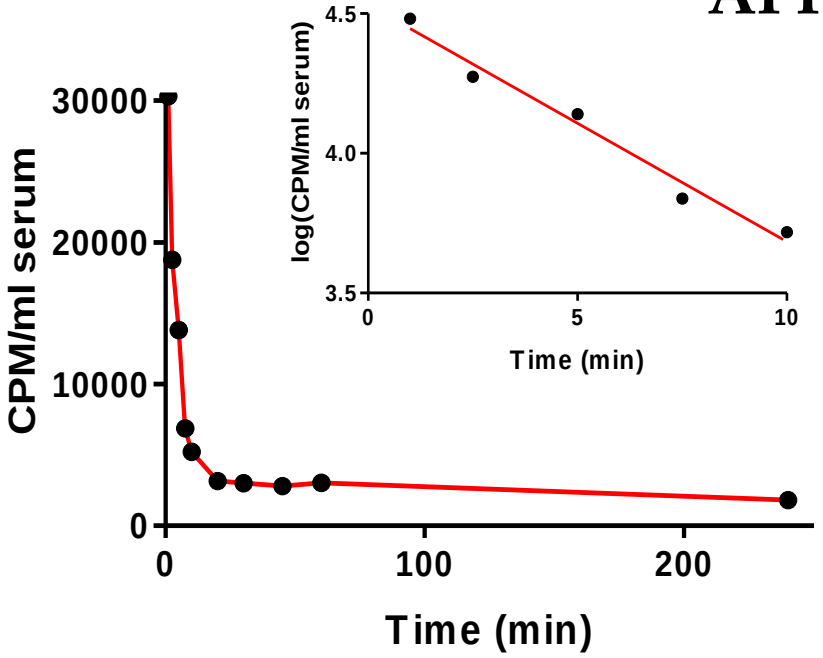
B = 

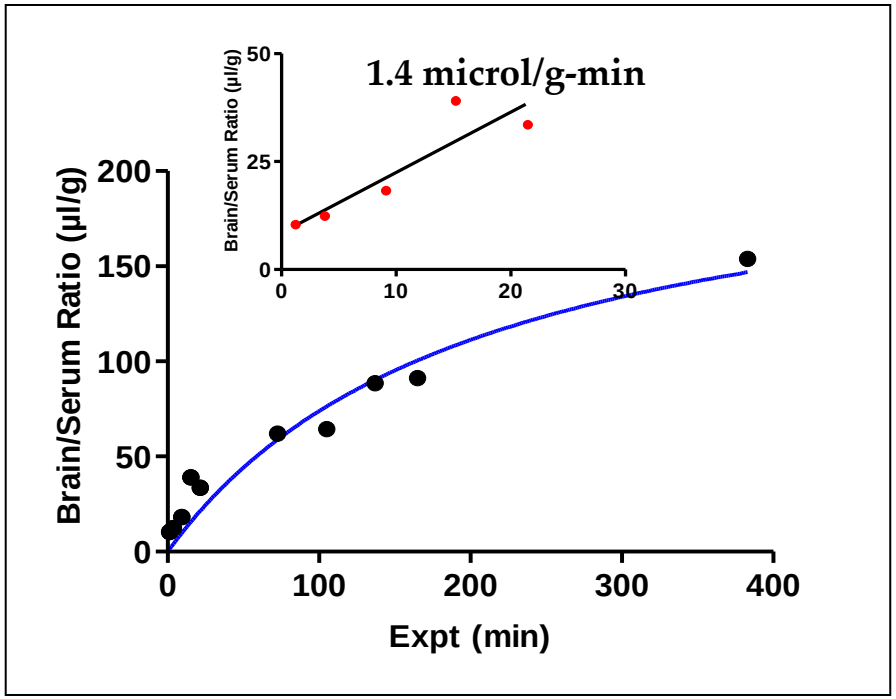


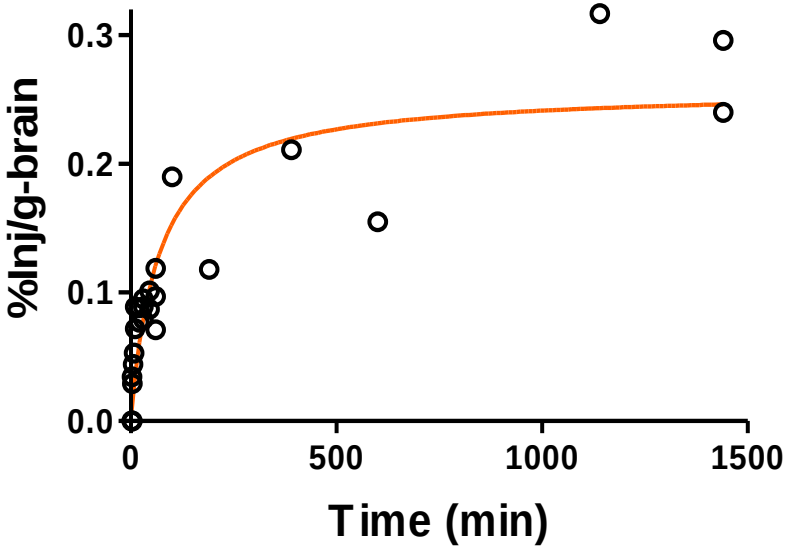
A = O(-): phosphodiester
= S(-): phorphorothioate

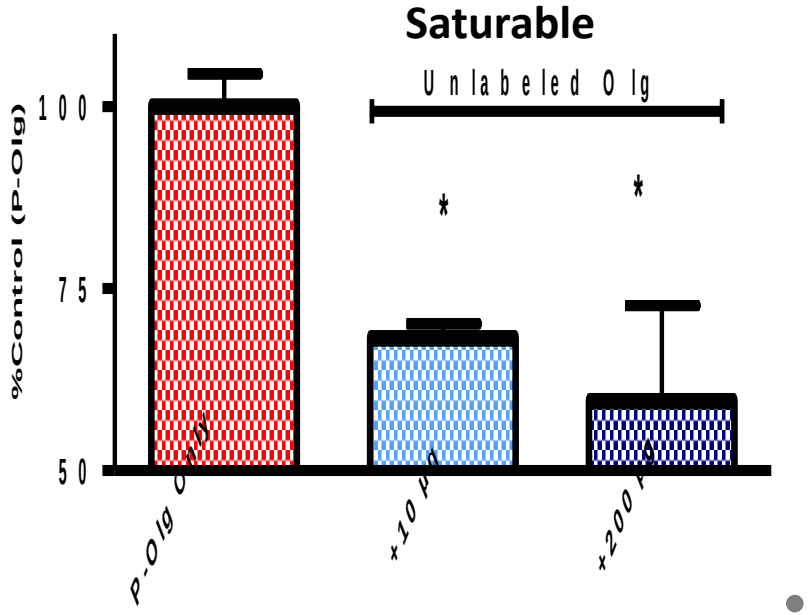
- Favorable PK
- Target Specificity
- Crosses the BBB

APP OPT Antisense



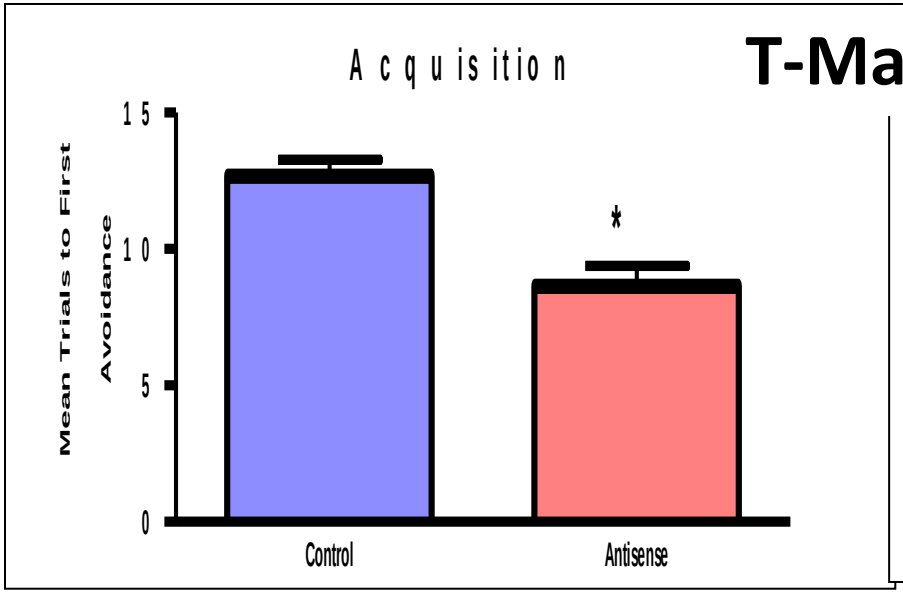


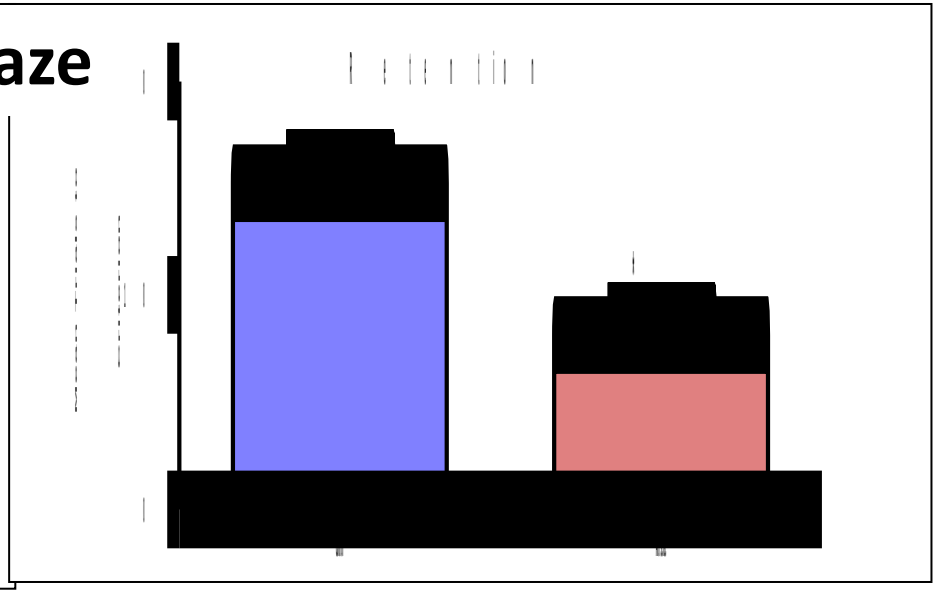


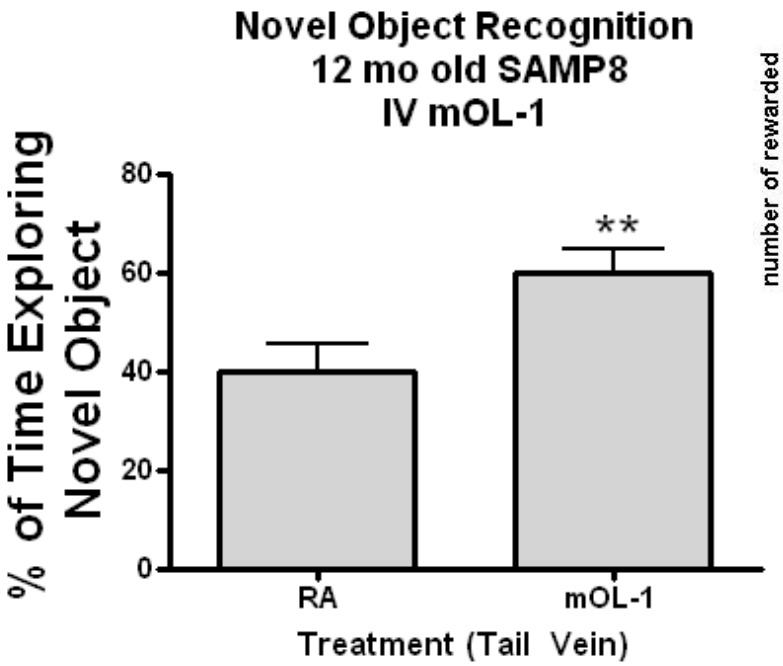


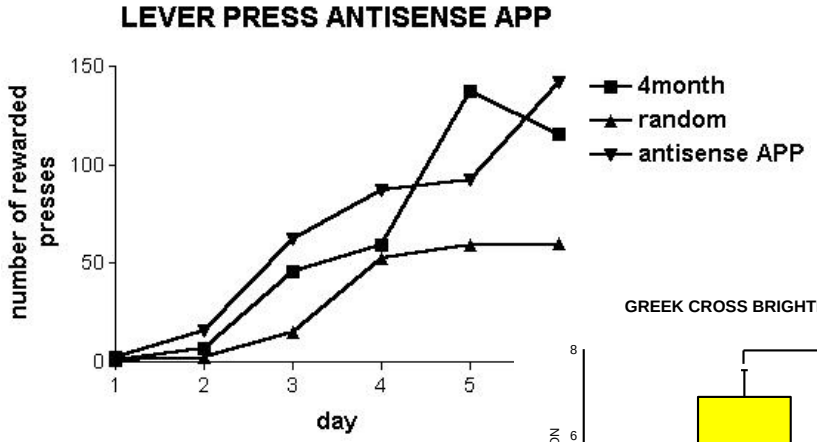
Banks, WA, J Pharmacol Exp Therap 297:1113-27, 01

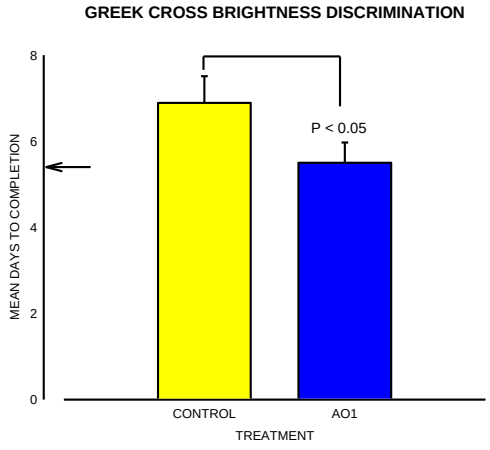
T-Maze


Acquisition
Mean Trials to First Avoidance
15
10
5
0
*
Control
Antisense




Novel Object Recognition
12 mo old SAMP8
IV mOL-1
% of Time Exploring Novel Object
80
60
40
20
0
**
RA
mOL-1
Treatment (Tail Vein)


LEVER PRESS ANTISENSE APP
number of rewarded presses
150
100
50
0
1
2
3
4
5
day
4month
random
antisense APP


GREEK CROSS BRIGHTNESS DISCRIMINATION
MEAN DAYS TO COMPLETION
8
6
4
2
0
P < 0.05
CONTROL
AO1
TREATMENT

Antisense Oligonucleotides

Protein Down Modulated	Result of Target Down Regulation
Amyloid Precursor Protein APP by EDN-OL1	• Restoration of cognitive function • Reduction of oxidative damage. • Restoration of normal efflux of amyloid proteins.
PACAP27 (PTS-6) Efflux transport protein: beta-F1 ATPase by EDN-OL202	• Increased PACAP27 in brain • Apparent increase in brain of peripherally administered PACAP27 • Neuroprotection in occlusive stroke model • Restoration of cognitive function in SAMP8 by antisense with co-administered PACAP27
Amyloid efflux transport protein: LRP-1 or low density lipoprotein receptor related protein-1	• Reduction of LRP-1 in brain • Reduction of amyloid efflux • Onset of cognitive dysfunction
Preproenkephalin	• Reduction of prepro & enkephalin. • Increased avidity for alcohol.

Pituitary Adenylate Cyclase Activating Polypeptide

Discovered by Akira Arimuar
(Tulane, New Orleans)
38 & 27 AA polypeptides in VIP/PACAP/Secretin Family
PACAP38 Powerful Neurotrohic
in vitro: fmol reverses gp120 neurotox
in vivo: iv injection reverses 4 vessels stroke
24 h after ischemia

PACAP27 Transported out of brain

PACAP 27 Brain-to-Blood Transporter: Beta-F1 ATPase (ATP synthase beta subunit)

Extra-membrane component of mitochondrial ATPase

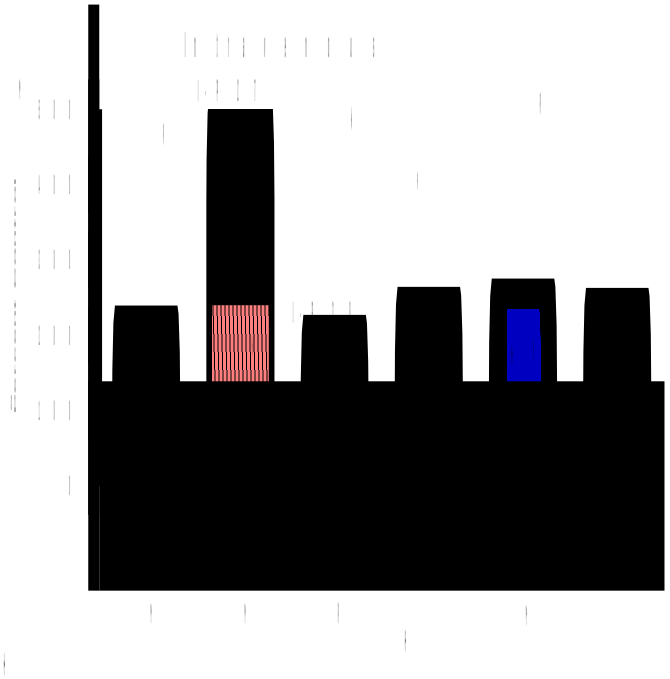
Apolipoprotein A-1 receptor: transports HDL into hepatocytes

Enterostatin (pentapeptide) receptor in neurons

Inhibiting Efflux Systems with Antisense

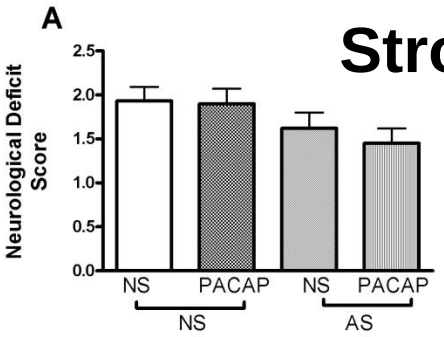
Rationale: Brain-to-blood Transporter for Pituitary Adenylate Cyclase Activating Polypeptide (PACAP27)

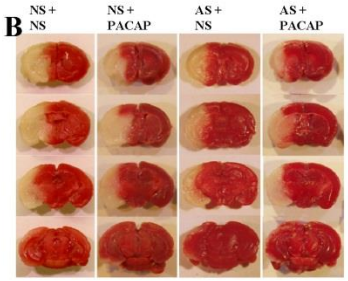
<u>Beta-F1 ATPase (ATP synthase beta subunit)</u>

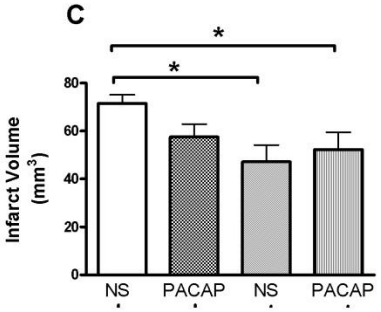


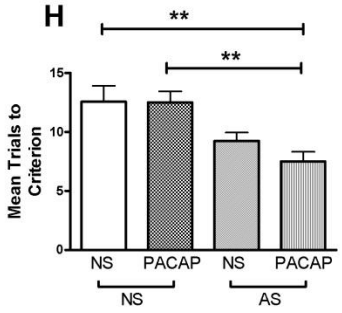
Dogrukol-Ak, JCBF&M: 29: 411-22, 2009

Stroke Model: MCAO-mouse







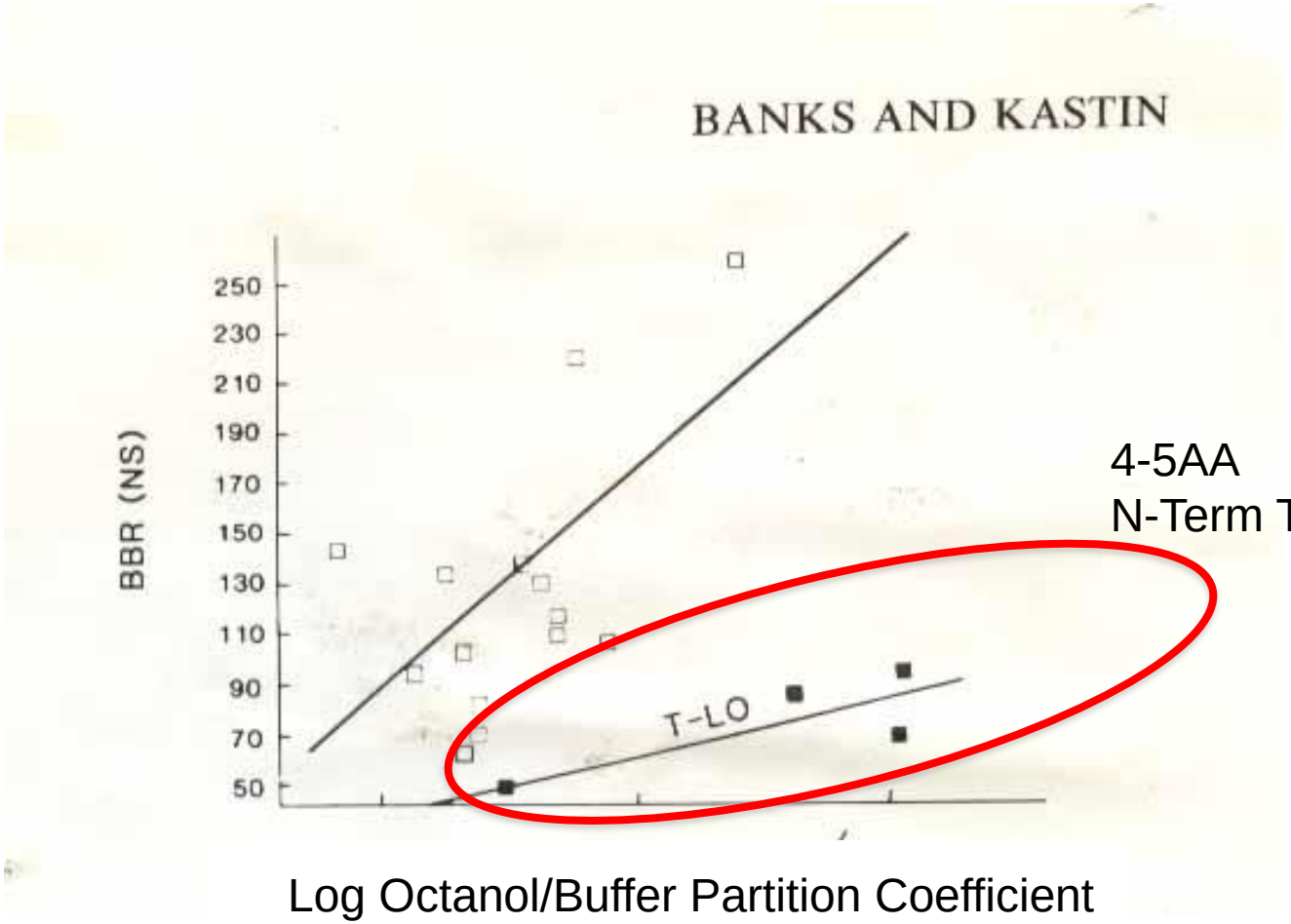


Alzheimer Model: SAMP8 mouse

Passive Diffusion

-Non-Saturable
- Lipid Solubility/ SQRT(MW)

Morphine
Ethanol
Nicotine



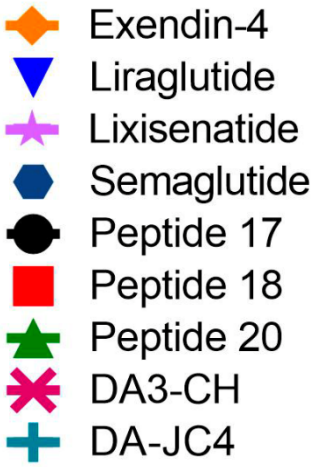
4-5AA
N-Term Tyrosine

Log Octanol/Buffer Partition Coefficient

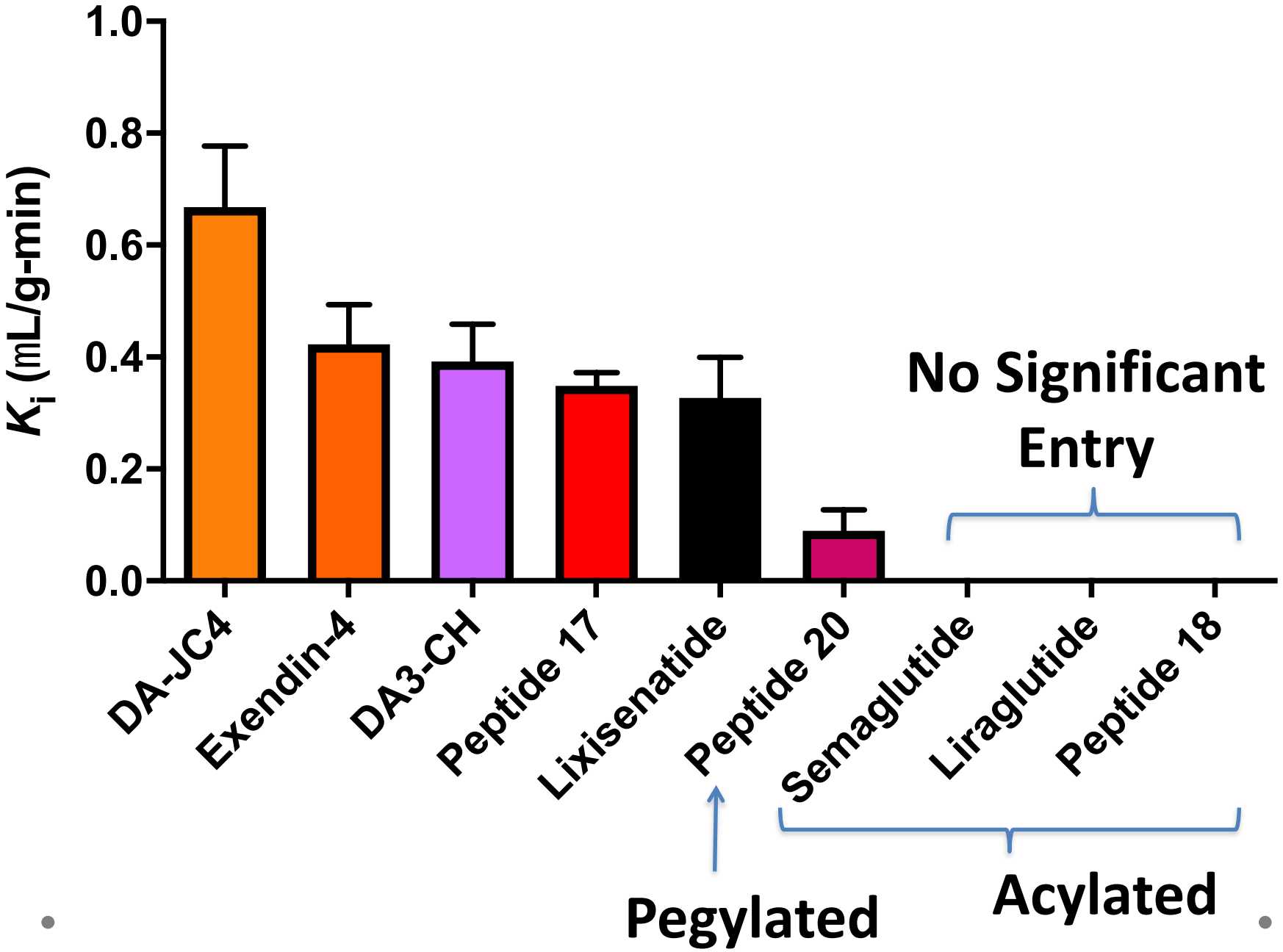
Measured BBB Permeability of 9 Incretin Peptides

Varying in

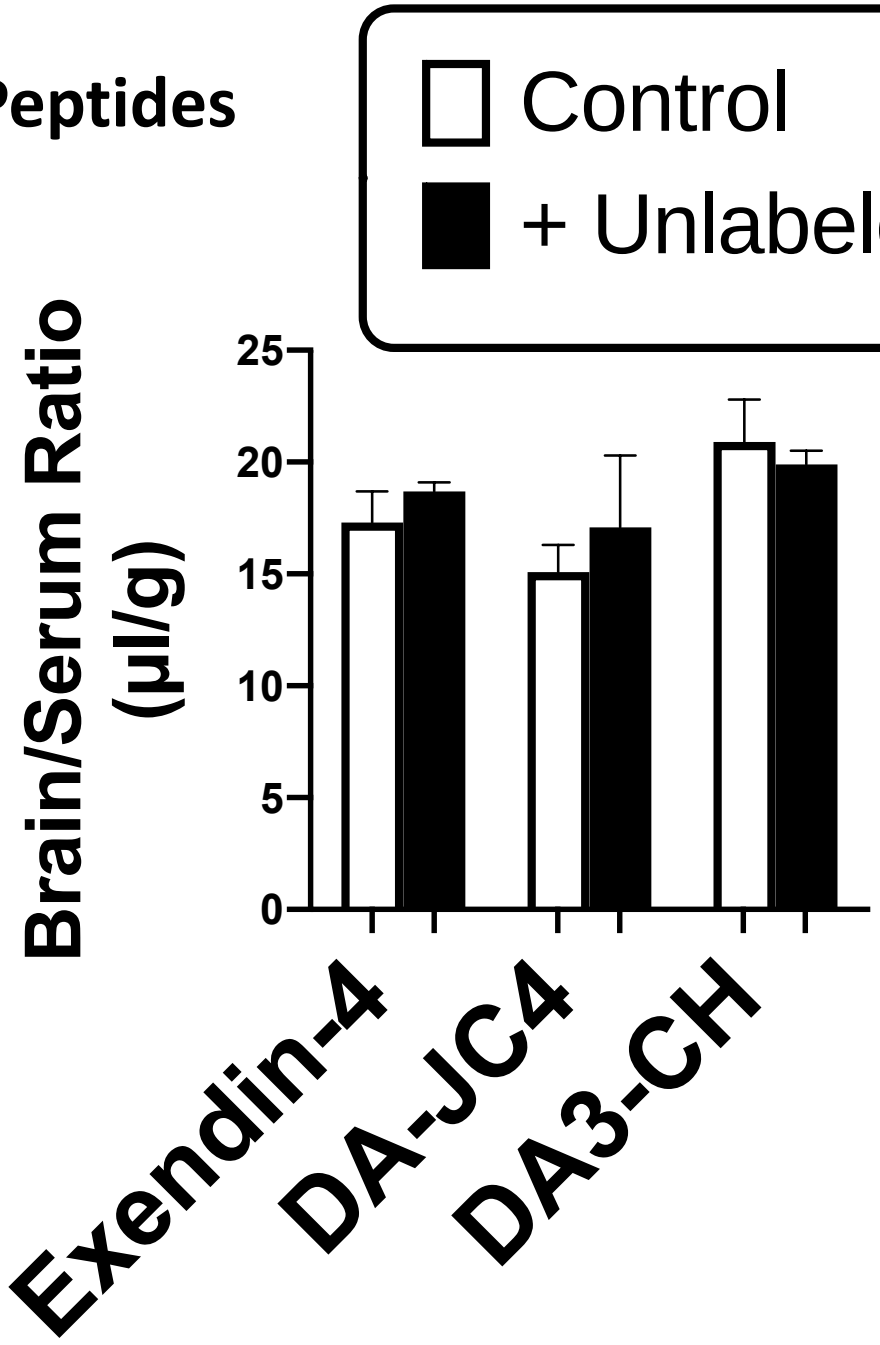
- **Acylation**
- **Pegylation**
- **Charge**
- **Lipid Solubility (-0.6 to -2.7)**
- **MW (3751-5420; 44,473)**
- **Single (GLP-1) vs Dual (GLP-1+GIP) Agonists**



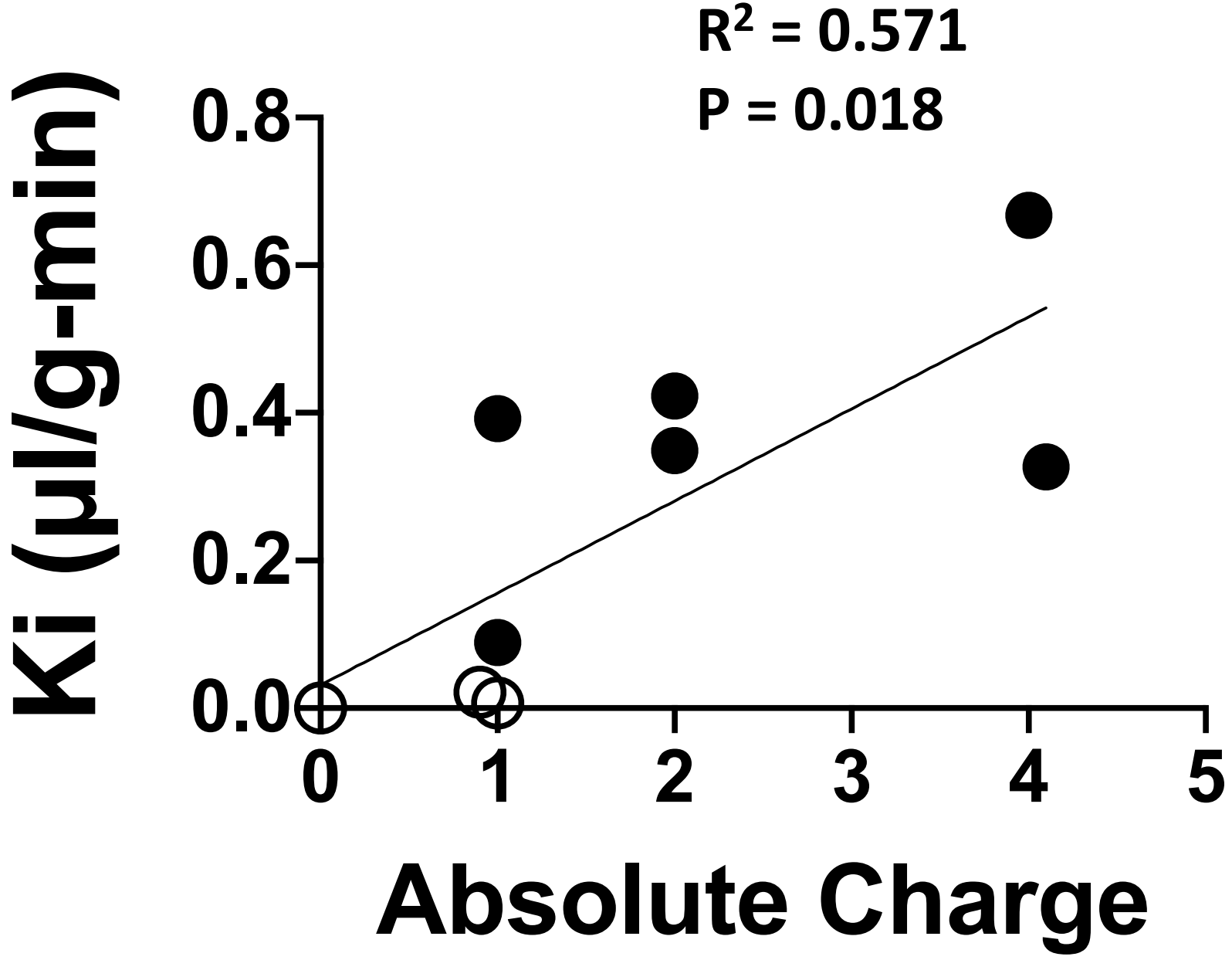
Salameh, TS et al Biochem Pharmacol, 2020
Rhea, EM Tissue Barriers, 2023


1.0
0.8
0.6
0.4
0.2
0.0
K_i (mL/g-min)
DA-JC4
Exendin-4
DA3-CH
Peptide 17
Lixisenatide
Peptide 20
Semaglutide
Liraglutide
Peptide 18
No Significant Entry
Pegylated
Acylated

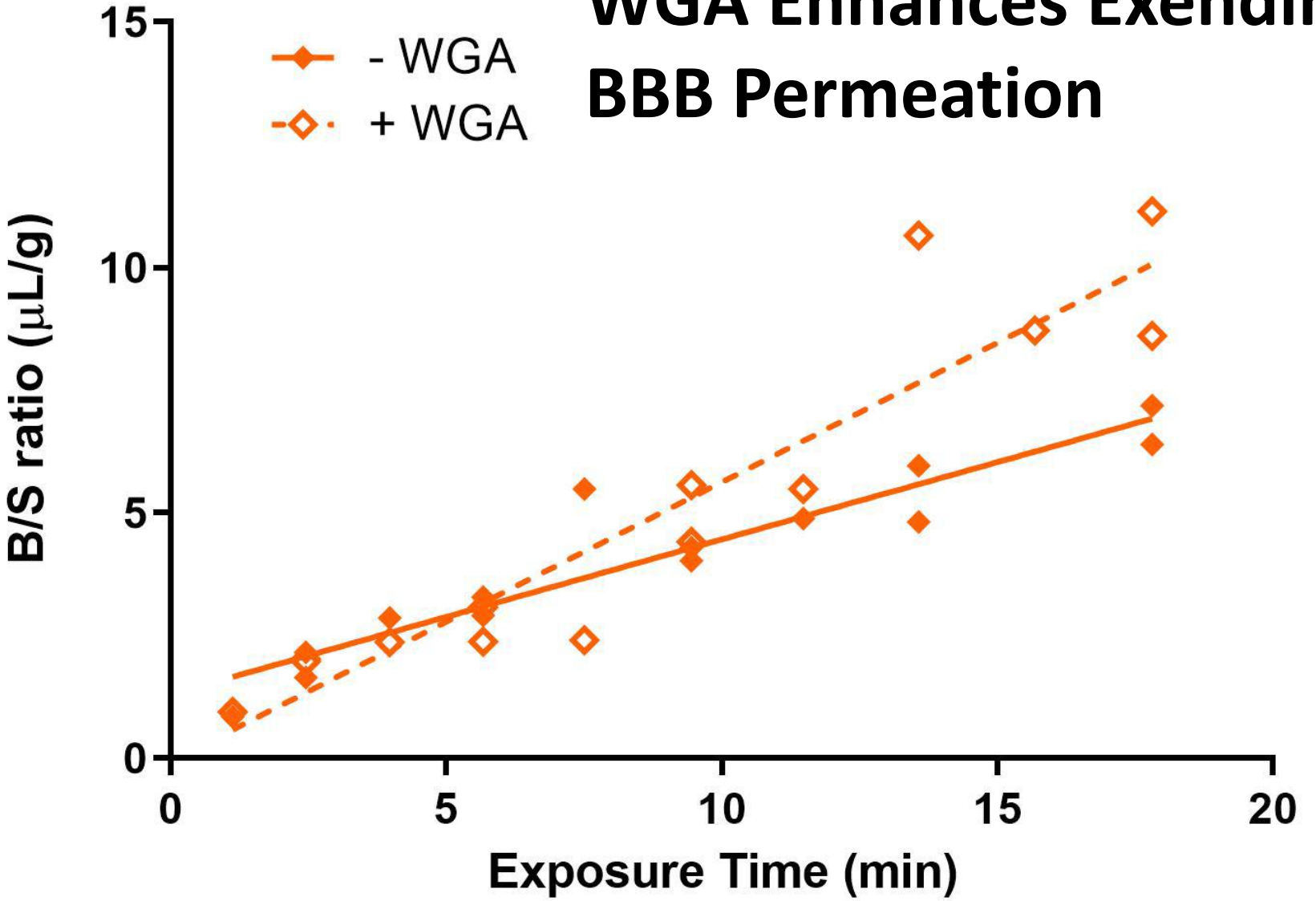
3 Fastest Peptides



NO SELF INHIBITION


R² = 0.571
P = 0.018
Ki (µl/g-min)
0.8
0.6
0.4
0.2
0.0
0
1
2
3
4
5
Absolute Charge

WGA Enhances Exendin-4 BBB Permeation



Mucopolysaccharidosis type VII
Sly Syndrome

Lysosomal storage disease:
Deficiency of ß-glucuronidase (GUS): 300 kDa tetramer

Results in accumulation of Glucosaminoglycans (GAGS) in brain and peripheral tissues

GUS internalized at cell surface by mannose 6-phosphate/insulin-like growth factor II receptor (M6P/IGF2R)

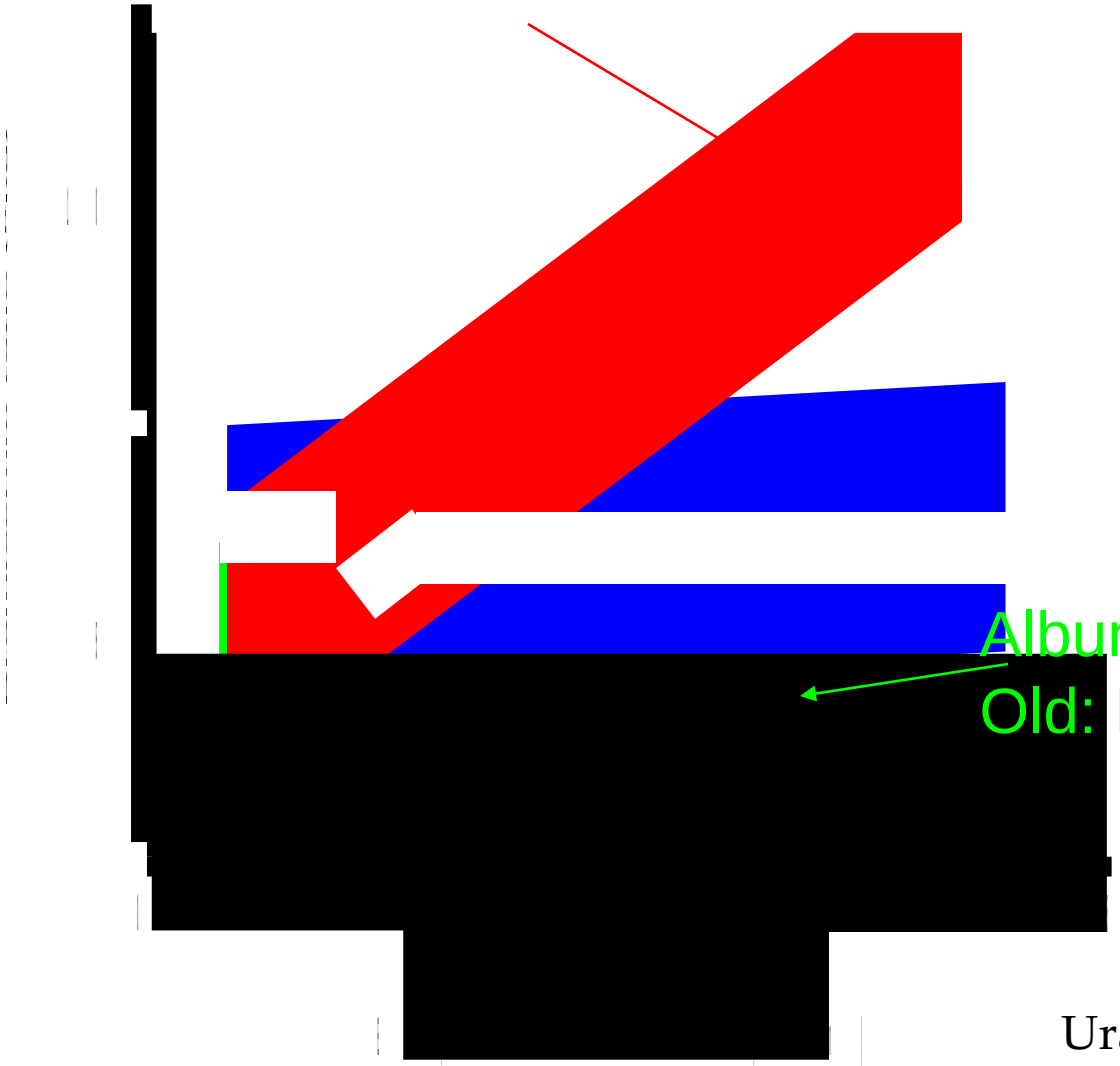
How to Deliver GUS to Brain?

Effects of IV GUS Treatment

	Peripheral Tissues	Brain
Neonates	Recovery	Recovery
Adults	Recovery	No Recovery

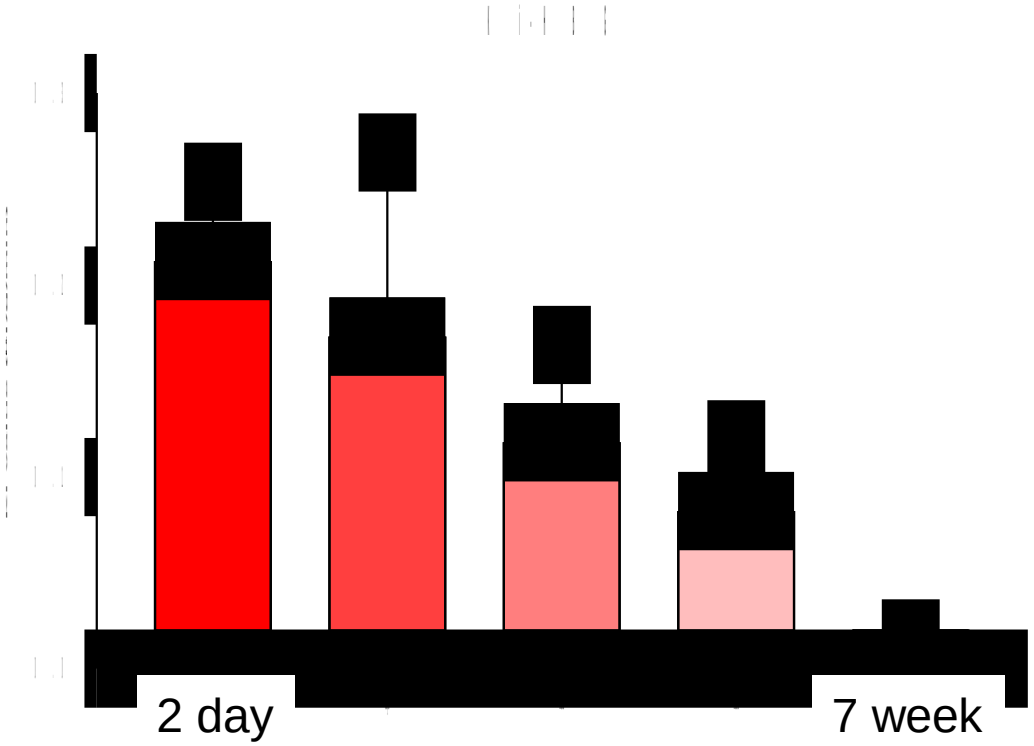
Leaky Neonatal BBB vs Saturable Transport System

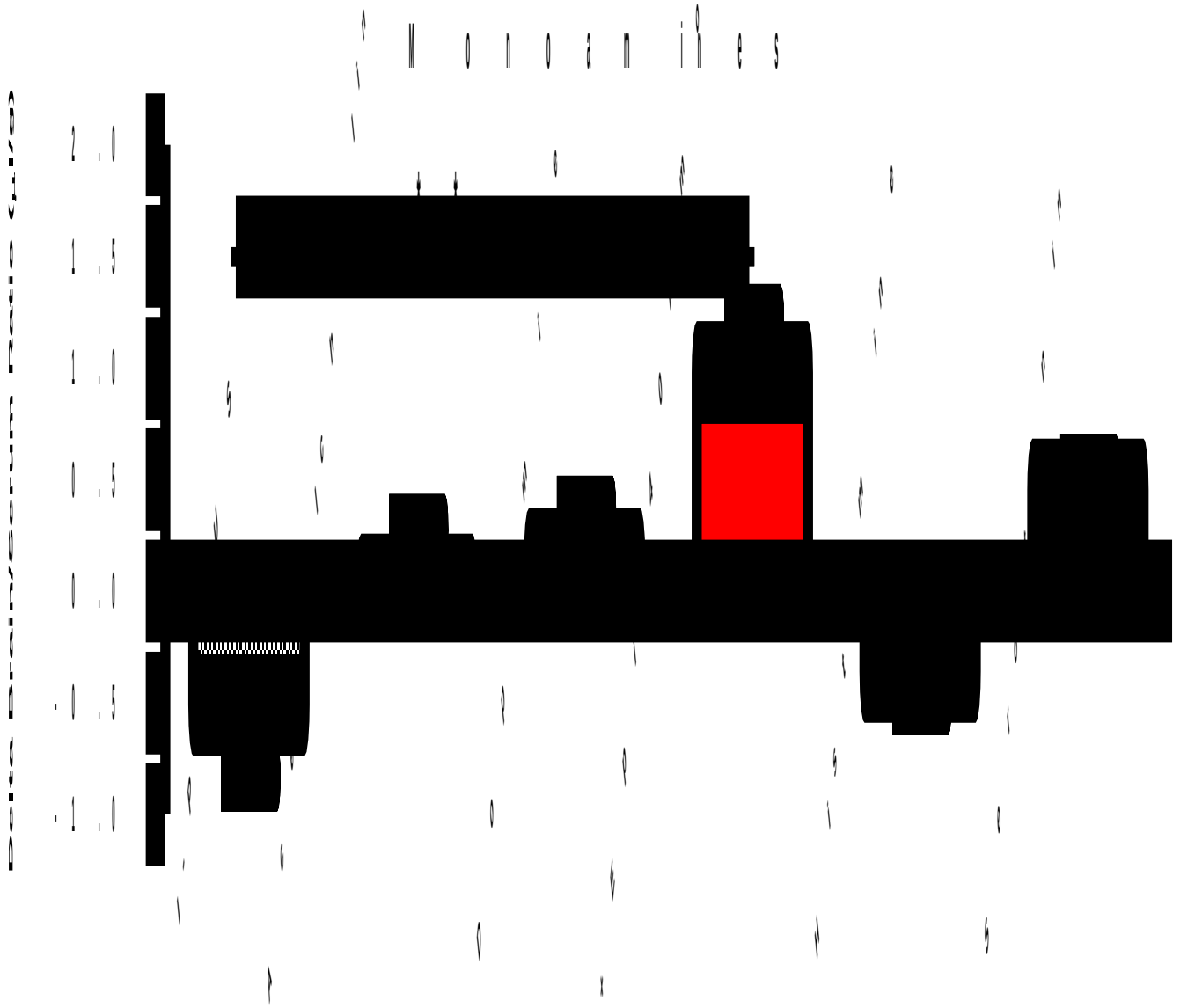
2 Day Old: GUSB Uptake (Ki = 0.3 µl/g-min)



Urayama et al
PNAS 101:12658-63

GUSB Transport Declines with Maturation





Urayama et al
PNAS 104:12873
PLOS One 10: e0142347
JCBF&M 36: 476

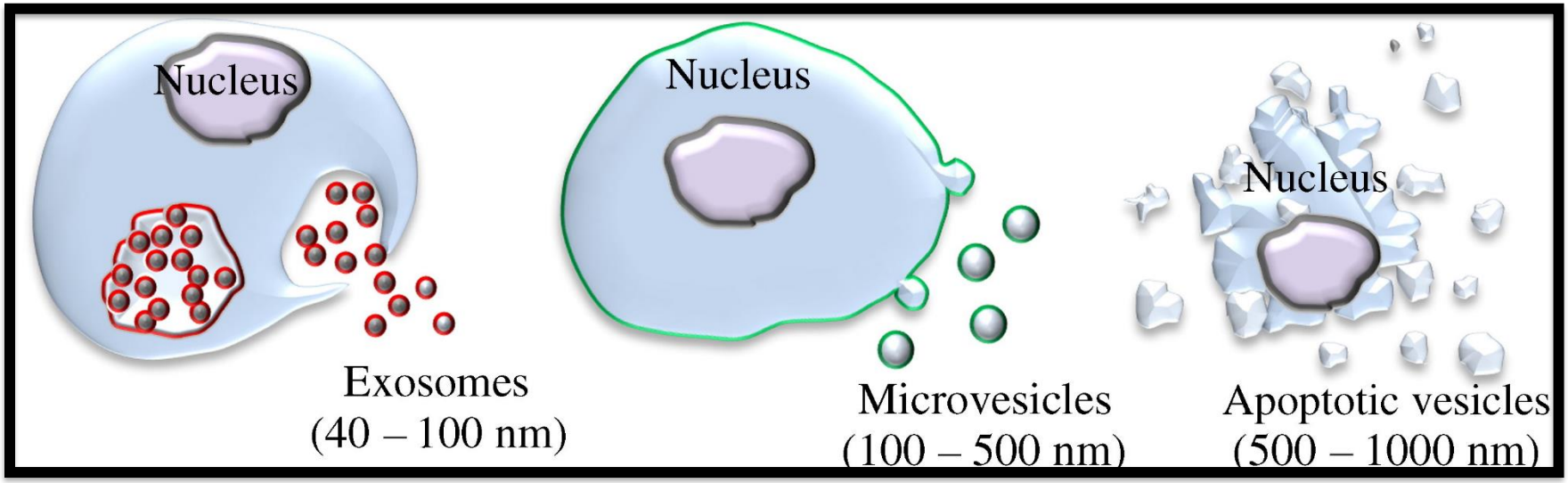
GUSB Findings

Transport by M6PR can be Re-Induced in the Adult with Epinephrine

Requires both Alpha$_1$ and Alpha$_2$ Adrenergic Activity

Effect is Immediate and Directly at the BBB

Extracellular Vesicles

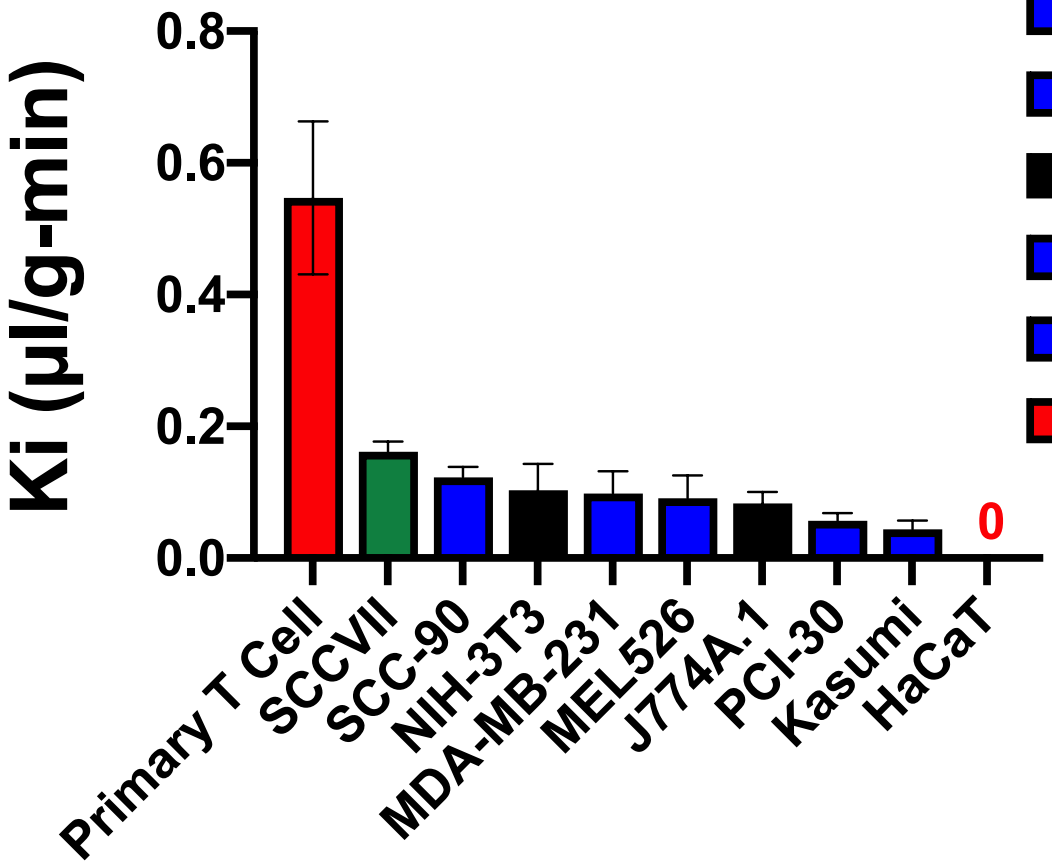


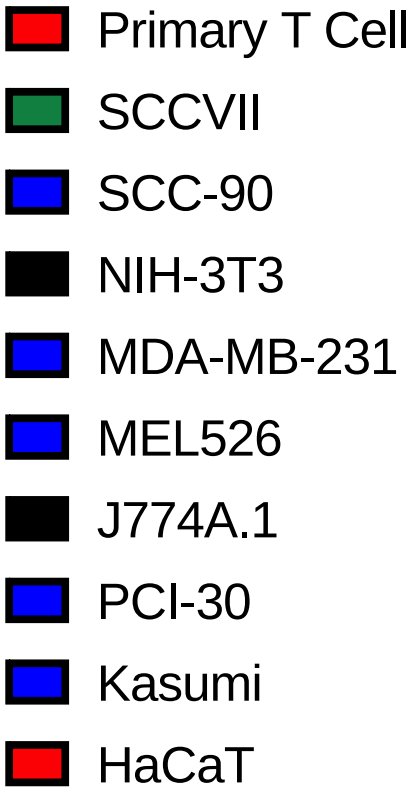
From:

Batrakova & Kim J Control Release 219: 396-405, 2015

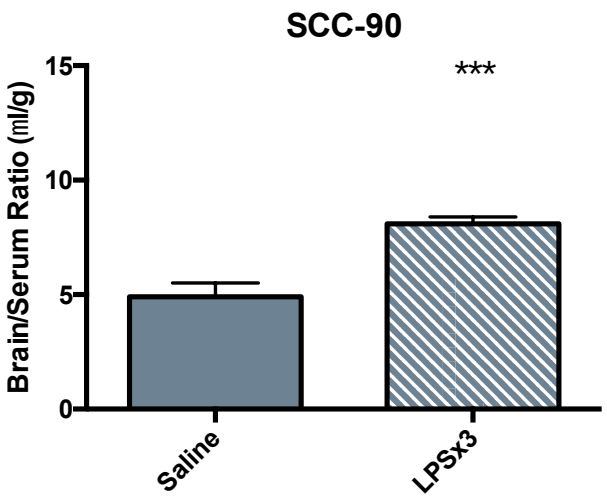
Exosomes Cross the BBB:

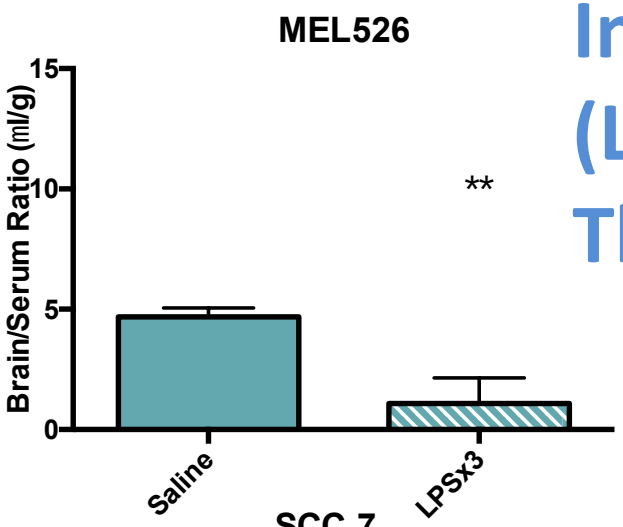
Black = Mouse Non-Cancer
Red = Human Non-Cancer
Green = Mouse Cancer
Blue = Human Cancer

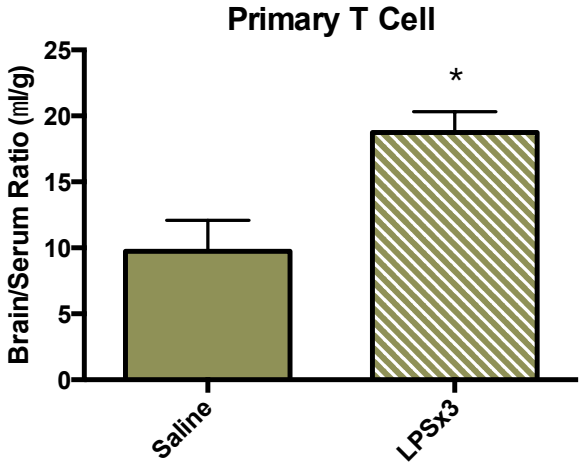


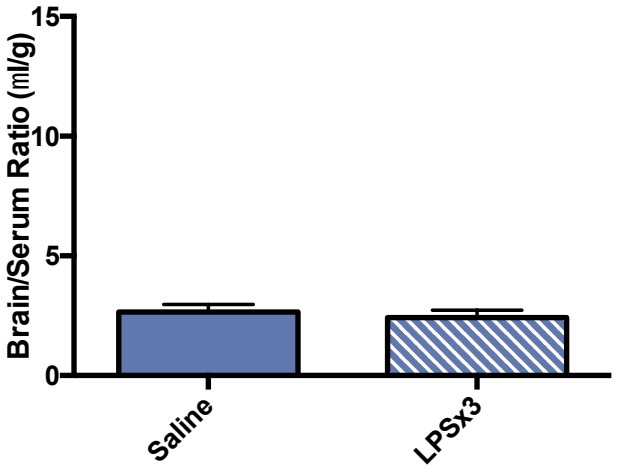


Banks et al
Int J Mol Sci
21:4407 '20





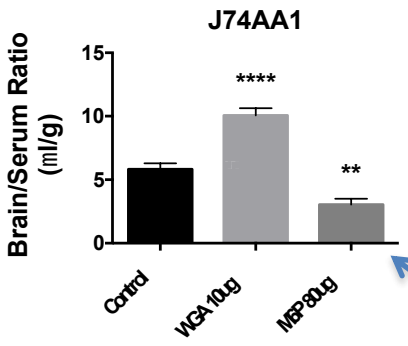


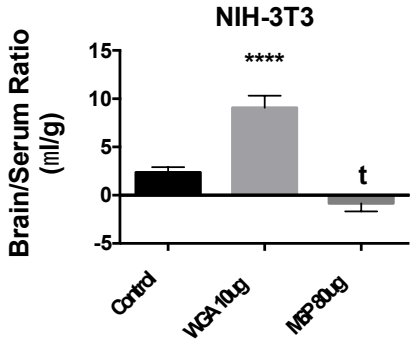


Inflammation (LPS) Three Patterns:

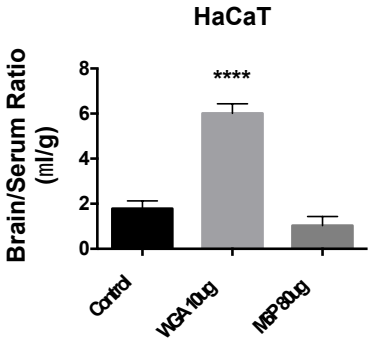
Increase: 6
Decrease: 1
No Change: 3

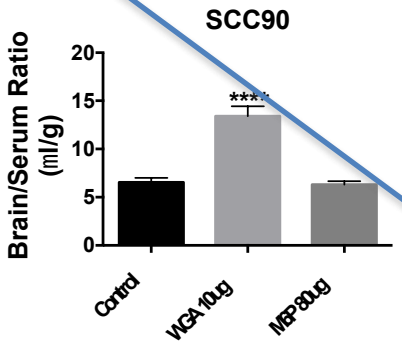
	SCC-90		MEL526		SCC-7		Primary-T Cell	
	Saline	LPSx3	Saline	LPSx3	Saline	LPSx3	Saline	LPSx3
Mean ± SEM	4.905 ± 0.601	8.098 ± 0.296	4.676 ± 0.375	1.081 ± 1.064	2.651 ± 0.318	2.423 ± 0.312	9.731 ± 2.372	9.000 ± 3.147
P value	0.0007		0.0046		0.6155		0.0119	



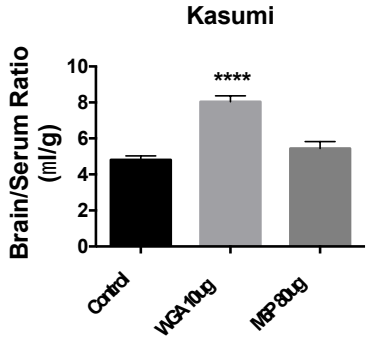


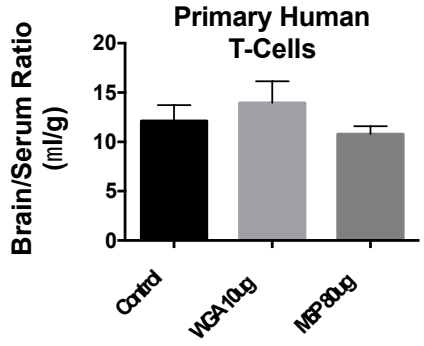
WGA Two Patterns:
Increase (5)
No Effect (5)





J74AA2 (Mu; Non-Ca)
Inhibited by M6P





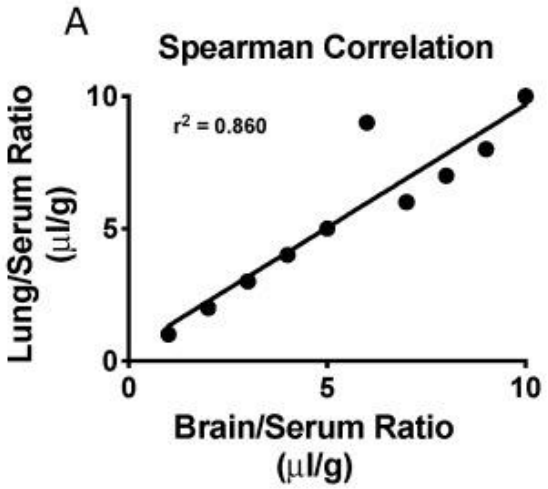
Treatment	Exosome Source	Lung	Brain	Kidney	Liver	Spleen
LPS	J774A.1	Decrease	No Change	No Change	Decrease	Decrease
LPS	NIH-3T3	No Change	No Change	No Change	Decrease	Decrease
LPS	Primary T Cell	Increase	Increase	Increase	No Change	Decrease
LPS	HaCaT	No Change	No Change	No Change	No Change	No Change
LPS	SCCVII	Increase	No Change	Decrease	Decrease	Decrease
LPS	MEL526	No Change	No Change	No Change	Decrease	Decrease
LPS	MDA-MB-231	Increase	Increase	No Change	No Change	Decrease
LPS	PCI-30	No Change	Increase	No Change	No Change	Decrease
LPS	SCC-90	Increase	Increase	Decrease	No Change	No Change
LPS	Kasumi	Increase	No Change	No Change	No Change	No Change
WGA	J774.1	Increase	Increase	Increase	Decrease	Decrease
WGA	NIH3T3	Increase	Increase	Increase	Decrease	Decrease
WGA	Primary T Cell	Increase	No Change	Increase	No Change	No Change
WGA	HaCaT	Increase	Increase	Increase	No Change	No Change
WGA	SCC-7	Increase	No Change	No Change	No Change	No Change
WGA	MEL526	Increase	No Change	No Change	No Change	Decrease
WGA	MDA-MB-231	Increase	No Change	No Change	Decrease	No Change
WGA	PCI-30	Increase	No Change	No Change	Decrease	Decrease
WGA	SCC-90	Increase	Increase	No Change	Decrease	Decrease
WGA	Kasumi	Increase	Increase	No Change	Decrease	Decrease

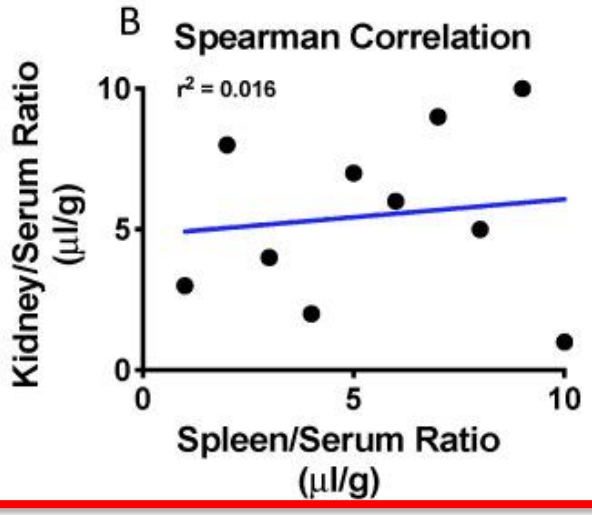
Increase

Decrease

No Change

Path Analysis

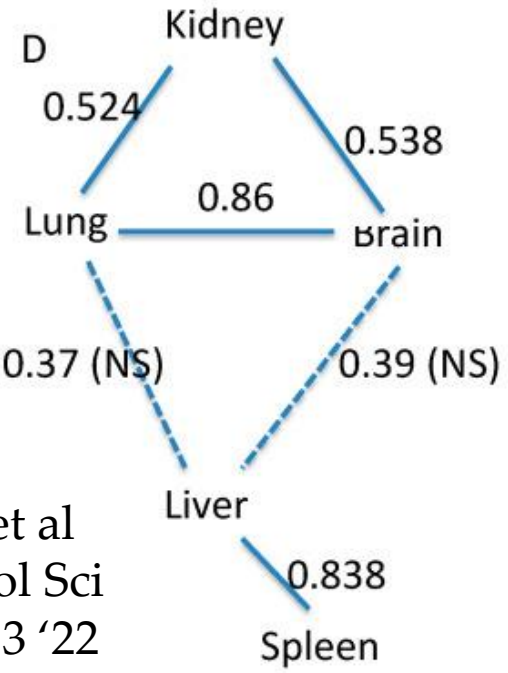




C

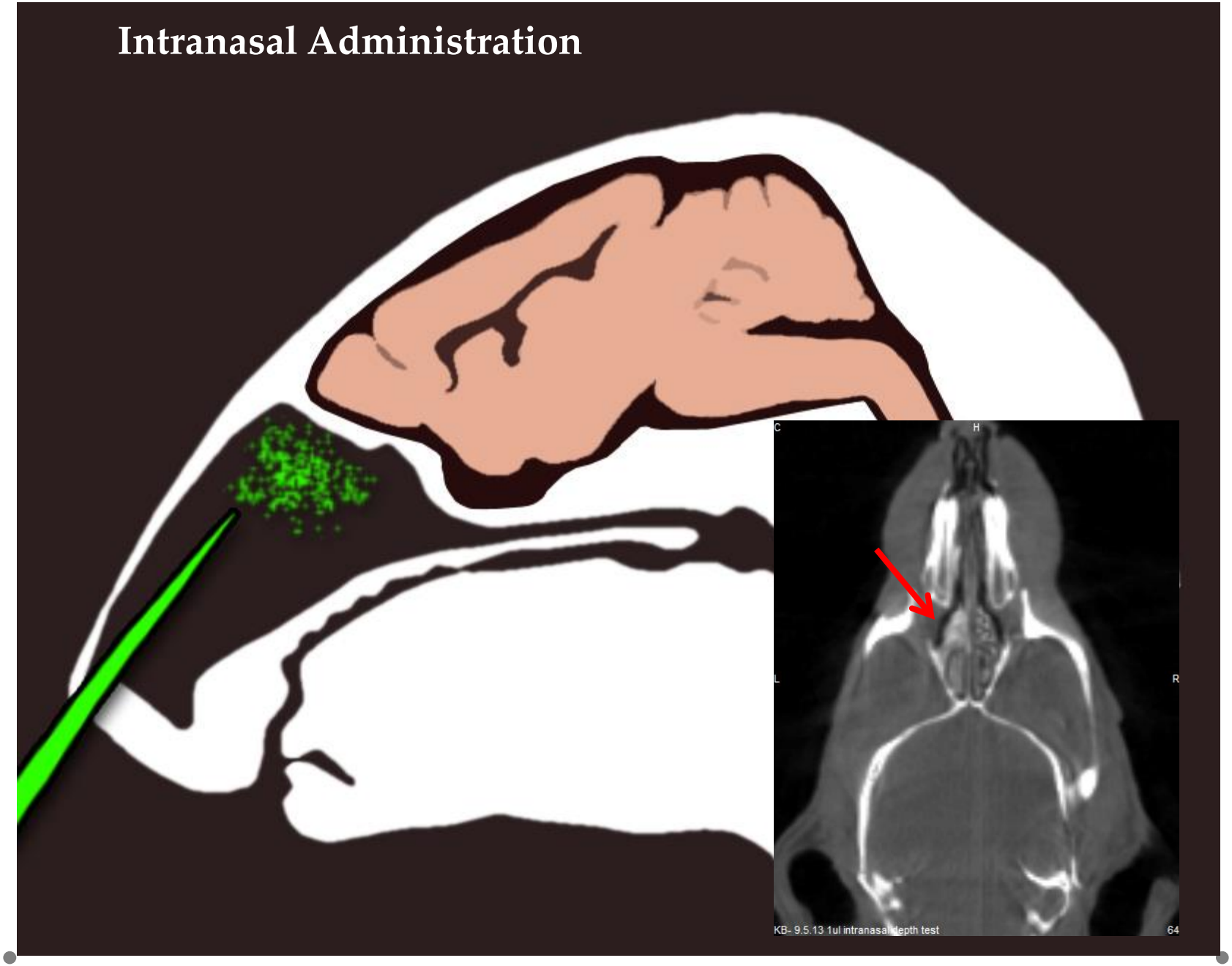
Tissues Correlated	r^2 value
BR-Lung	**0.86** *
Liv-Sp	**0.838** *
Br-Kid	**0.538** *
Lung-Kid	**0.52** *
Br-Liv	0.39
Liv-Lung	0.375
BR-Sp	0.207
Liv-kid	0.196
Lung-Sp	0.185
Kid-Sp	0.016

*p<0.05

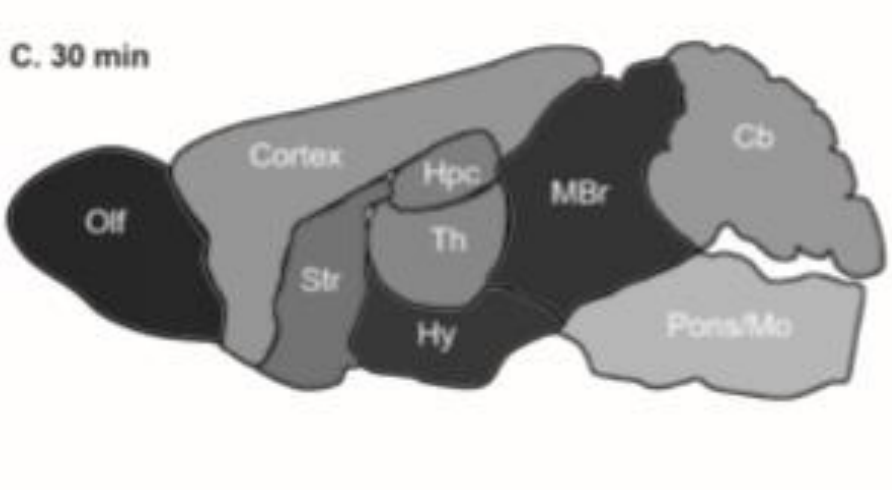


Banks et al
Int J Mol Sci
23:12513 '22

Intranasal Administration



Insulin Levels 30 min After INL Delivery

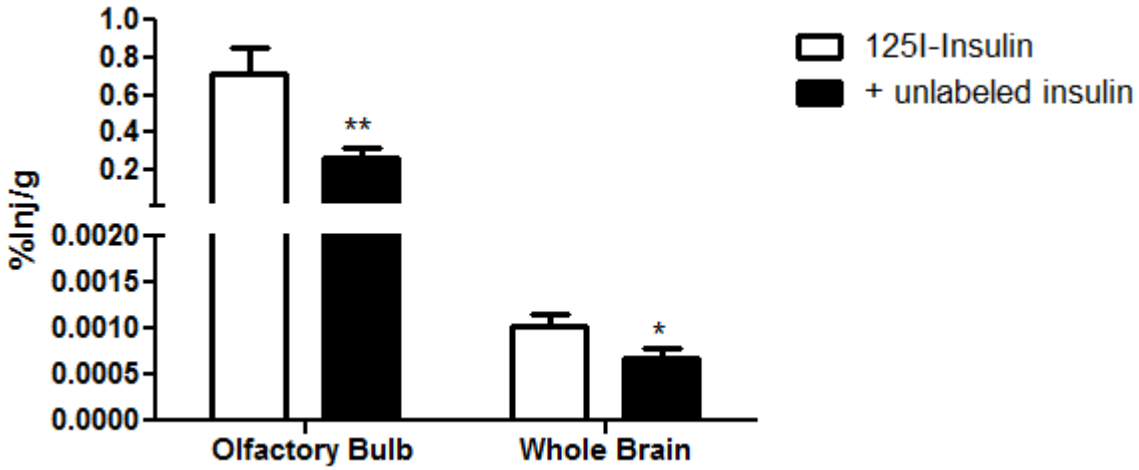


%Injection per gram

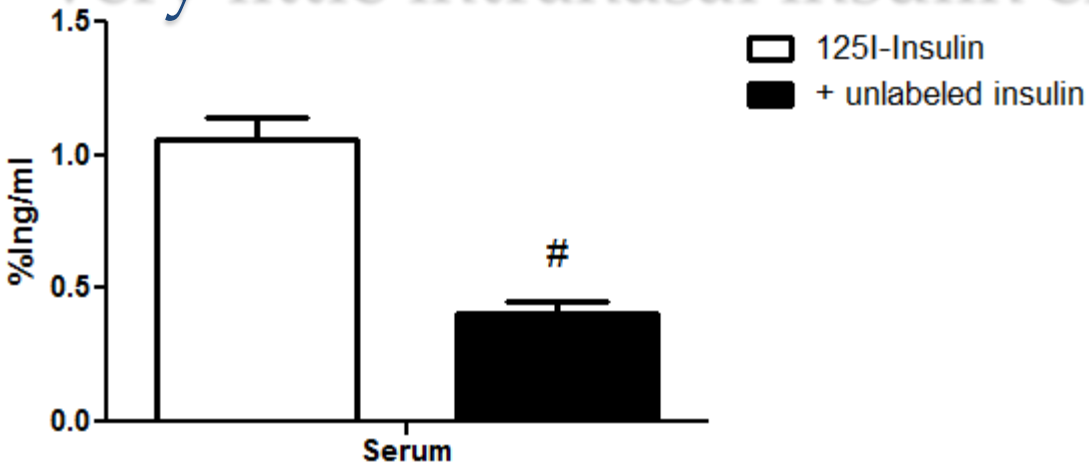
Region	Abbreviation	30 min
Olfactory Bulb	Olf	0.288 ± 0.070
Cortex	Cortex	0.162 ± .023
Striatum	Str	0.209 ± 0.052
Hypothalamus	Hy	0.265 ± 0.071
Hippocampus	Hpc	0.193 ± 0.046
Thalamus	Th	0.182 ± 0.021
Cerebellum	Cb	0.158 ± 0.017
Midbrain	MBr	0.271 ± 0.054
Pons/Medulla	Pons/Mo	0.121 ± 0.019

Rhea et al *JAD* 2017

Intranasal insulin transport is saturable

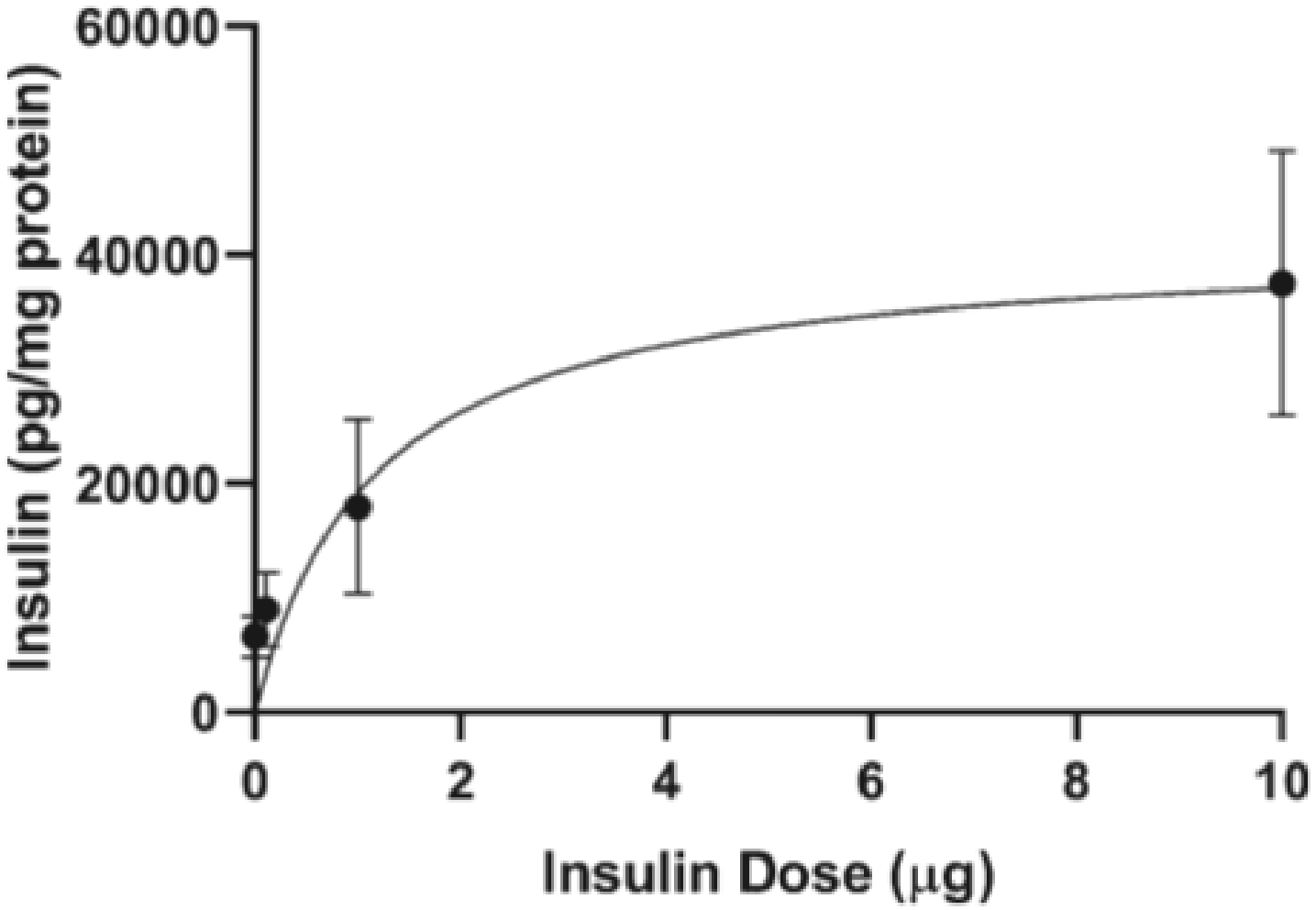


Very little intranasal insulin enters the blood

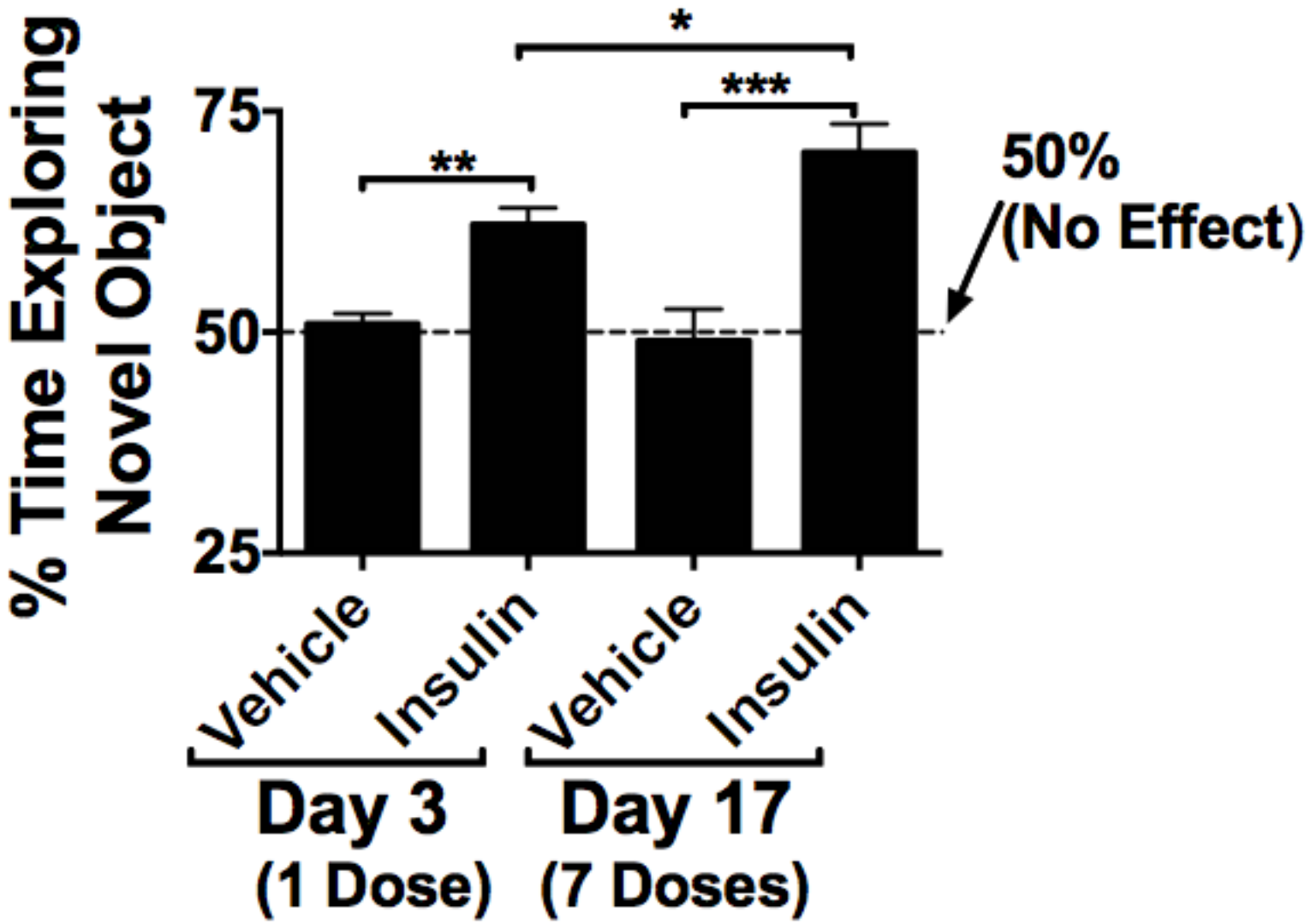


n =46-48; * $p<0.05$, ** $p<0.01$, # $p<0.001$; $4x10^5$ cpm/mouse

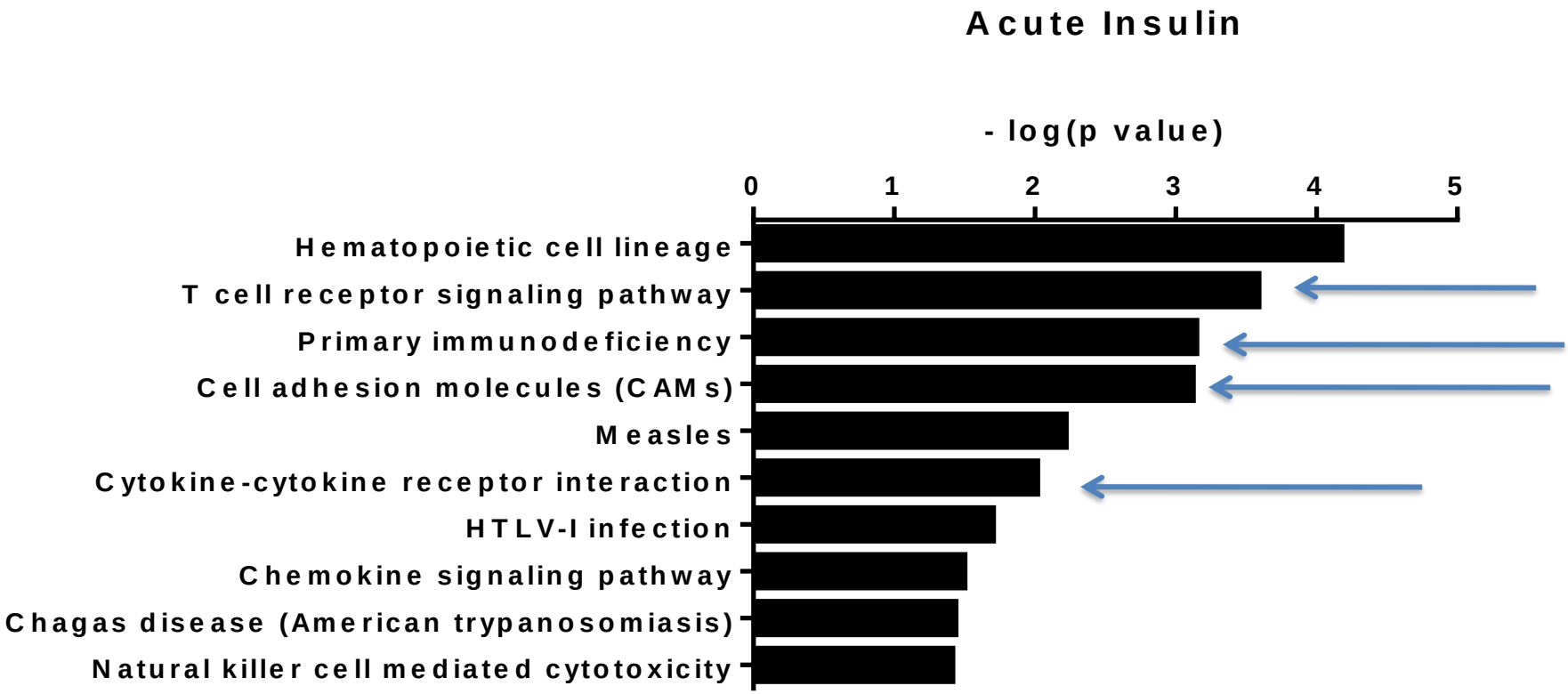
Human Intranasal Insulin: Dose vs Hippocampal Concentration



Memory:
Object Recognition



Top 10 Pathways Changed in Aged +/- Insulin



AGE

Table. T Cell Receptor Signaling Pathway Genes

Gene	Gene Symbol	LogFC	P Value	Effect of Age
CD3 antigen, delta polypeptide	Cd3d	6.484	0.0261	Up
CD3 antigen, gamma polypeptide	Cd3g	5.449	0.0231	Up
CD3 antigen, epsilon polypeptide	Cd3e	4.378	0.0380	Up
CD247 antigen	Cd247	3.281	0.0030	Up
CD28 antigen	Cd28	3.075	0.0007	Up
linker for activation of T cells	Lat	1.787	0.0106	Up
programmed cell death 1	Pdcd1	1.425	0.0013	Up
CD4 antigen	Cd4	1.365	0.0348	Up
protein tyrosine phosphatase, receptor type, C	Ptprc	1.167	0.0015	Up
lymphocyte protein tyrosine kinase	Lck	1.135	0.0282	Up

Acute

While there were 15 genes that fit this pathway, only the Top 10 genes affected by age based on LogFC are reported here

CONCLUSIONS

Many Biologics (& their analogs) DO Cross the BBB

- **In Amts that Affect Brain Function**
- **Saturable**
- **Passive Diffusion**
- **Adsorptive Transcytosis (?)**

Peptides

Regulatory Proteins

Antisense Molecules (Phosphorothioates)

Some Lysosomal Enzymes (e.g. GUSB)

- **Neonatal - M6PR**
- **Inducible – alpha1 and 2 adrenergics**

Exosomes

Intranasal Route (Insulin)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Rachel Knopp
Lindsey Williams
Kristen Baumann
Riley Weaver
Taka Fujimoto
Shelly Erickson
Cassidy Noonan
Elizabeth Rhea
May Reed

Friends, Colleagues, & Graduate Students

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Sue Farr

Lawrence Maness

Abba Kastin

Naoko Nonaka

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