Parabiosis experiments

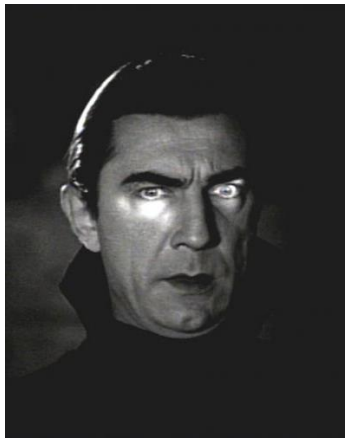
1st technical journal club
Johanna Schaffenrath
Division of Neurosurgery

“It may sound like vampirism, but the bizarre practice known as ‘parabiosis’ has caught the attention of many life-extension enthusiasts – including billionaire tech investor Peter Thiel.”

DailyMail, 01.08.2016

http://www.dailymail.co.uk/sciencetech/article-3718758/Peter-Thiel-believes-blood-transfusions-young-key-living-forever.html#ixzz4S3N3tyZm

Peter Thiel



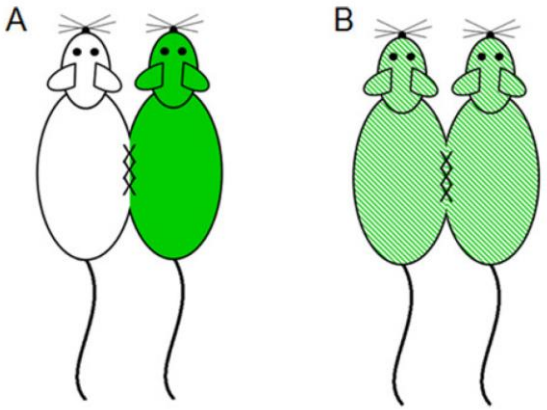
- Peter Thiel (49)
- German-American
- Hedge fund manager, author, …
- Via Thiel Capital Management involved in PayPal, Facebook, Clarium Capital …
- Fortune: $2.7 billion
- Investment in «Ambrosia» trial: Healthy aged (35+) get blood plasma from donors under 25
- “It’s this extremely abundant therapeutic that's just sitting in blood banks”
- “It may sound like vampirisim, but the bizarre practice known as ‘parabiosis’ has caught the attention of many life-extension enthusiasts – including billionaire tech investor Peter Thiel.”

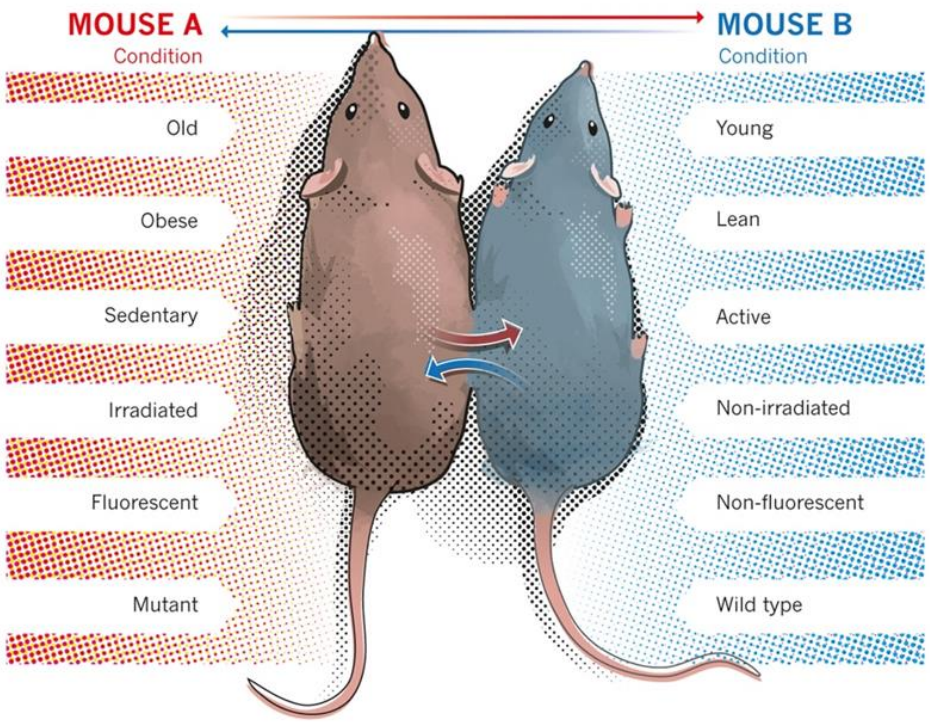
http://www.sciencemag.org/news/2016/08/young-blood-antiaging-trial-raises-questions

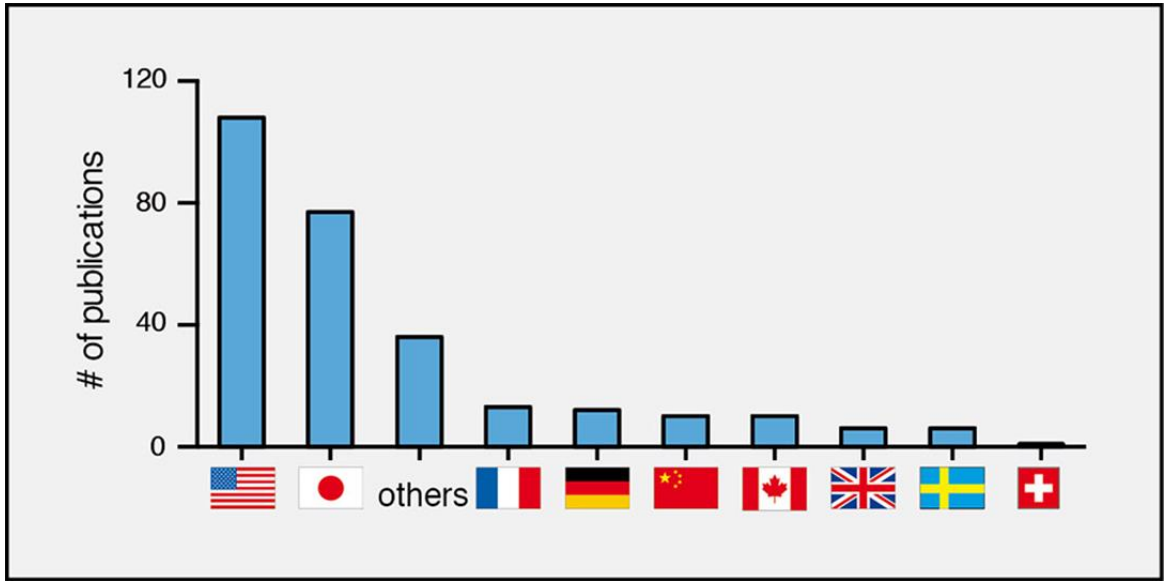
http://www.dailymail.co.uk/sciencetech/article-3718758/Peter-Thiel-believes-blood-transfusions-young-key-living-forever.html#ixzz4S3N3tyZm

Facts

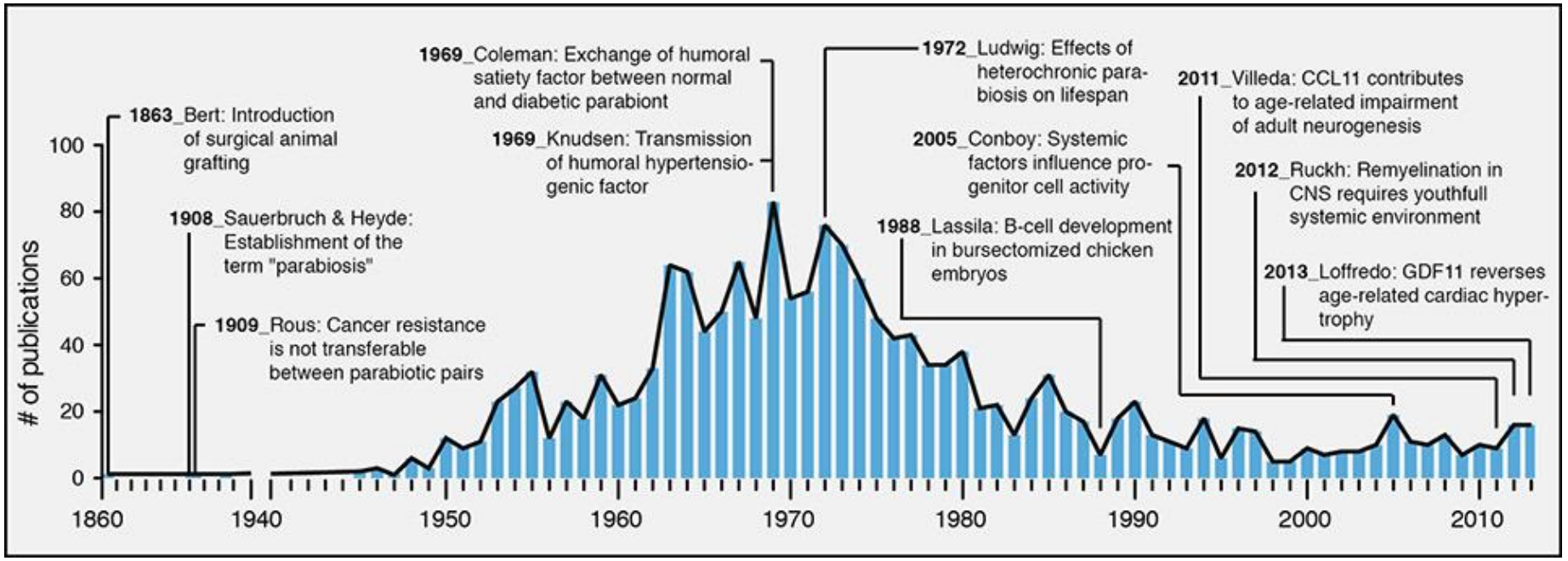
- Unites vasculature of living organisms
- Mimics conjoined twins
- First practiced in 1860 by Paul Bert
- App. 20 dps shared blood supply occurs







http://www.gopubmed.org/web/gopubmed/



http://www.gopubmed.org/web/gopubmed/ ; Nik Spencer/Nature; Chart Data: A. Eggel & T. Wyss-Coray Swiss Med. Wkly 144, W13914 (2014)

Paul Bert`s technique

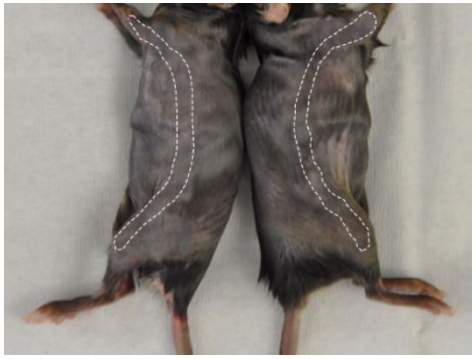
- Surgical joining of two organisms through joining skin and muscular walls of two rats
- To study shared circulatory systems (hormone studies, progenitor cells in neovascularization, hematopoietic stem cell migration…)
- Partnered animals share circulating antigens -> no triggering of immunological reactions (male/female joints don’t show H-Y AB)

- Originally Bert joined skin and muscle walls
- Caused significant strain and high mortality (infections)

Modern techniques

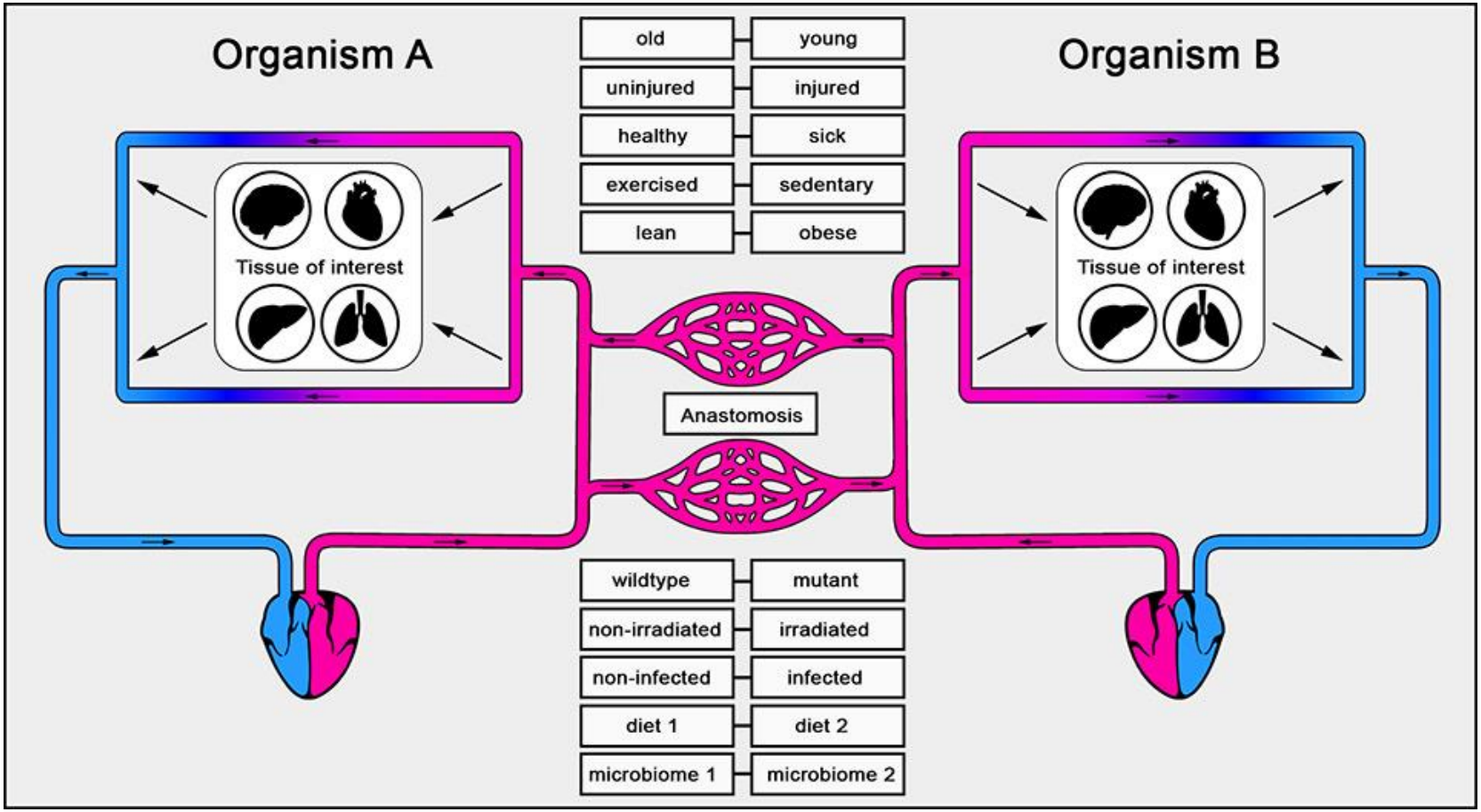
- Today Bunster & Meyer (1933) method of choice
- Joining scapula joints body cavities and skin (less pain, minimal post operative care & reduced mortality)
- Modifications: less invasive and firmer joining
- Mice connected through the elbow and knee joints and skin (prevents extension of skin and less pain and complications)
- Procedure remains invasive
- Animals receive a combination of antibiotics
- monitored regularly

Parabiosis surgery

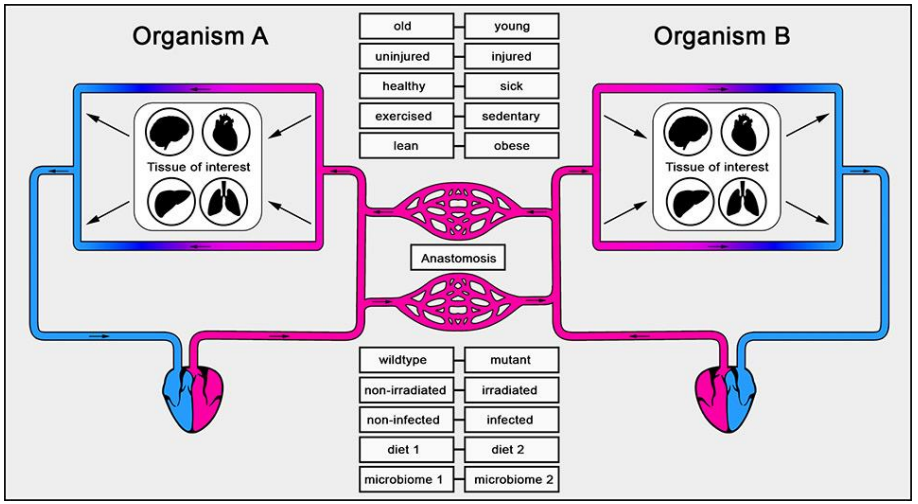


- Mice with similar genetic background, similar weight and size -> monitor 2 weeks min.
- Analgesia administration
- Join animals
- Skin incisions, elbow and knee joining, connect skin of animals, administer NaCl to prevent dehydration
- Keep administer analgesics
- Monitoring of animals daily for two weeks
- Prevent bacterial infections
- Blood chimerism occurs 10-14 dps

Parabiosis in Mice: A Detailed Protocol Paniz Kamran, Konstantina-Ioanna Sereti, Peng Zhao, Shah R. Ali, Irving L. Weissman, Reza Ardehali


Organism A
Organism B
old
young
uninjured
injured
healthy
sick
exercised
sedentary
lean
obese
Tissue of interest
Tissue of interest
Anastomosis
wildtype
mutant
non-irradiated
irradiated
non-infected
infected
diet 1
diet 2
microbiome 1
microbiome 2

Post-OP Study



- Asses the physiological changes

of parabionts

- Joined skin and subcutaneous tissues
- Physiological, social, affective life: observation for 120 dps
- 2-3 dps animals suffered from severe pain and distress
- following days/weeks mice began to recover & displayed behavioral adaptations
- All animals survived day 120
- At 3 dps, the body weight decreased, but recovered until 30 dps
- Normal blood exchange rate post op (good behaviorally adaption to the parabiotic situation)

nature medicine

Young blood reverses age-related impairments in cognitive function and synaptic plasticity in mice

Saul A Villeda[1–6], Kristopher E Plambeck[1,2,10], Jinte Middeldorp[6,10], Joseph M Castellano[6,10], Kira I Mosher[6,7,10], Jian Luo[6], Lucas K Smith[1,2], Gregor Bieri[1,2,6,7], Karin Lin[1–3], Daniela Berdnik[6], Rafael Wabl[6], Joe Udeochu[1,2,4], Elizabeth G Wheatley[1,2,5], Bende Zou[8], Danielle A Simmons[6], Xinmin S Xie[8], Frank M Longo[6] & Tony Wyss-Coray[6,7,9]

Research

JAMA Neurology | Original Investigation

Preclinical Assessment of Young Blood Plasma for Alzheimer Disease

Jinte Middeldorp, PhD; Benoit Lehallier, PhD; Saul A. Villeda, PhD; Suzanne S. M. Miedema, MSc; Emily Evans; Eva Czirr, PhD; Hui Zhang, PhD; Jian Luo, MD, PhD; Trisha Stan, PhD; Kira I. Mosher, PhD; Eliezer Masliah, MD, PhD; Tony Wyss-Coray, PhD

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Aim

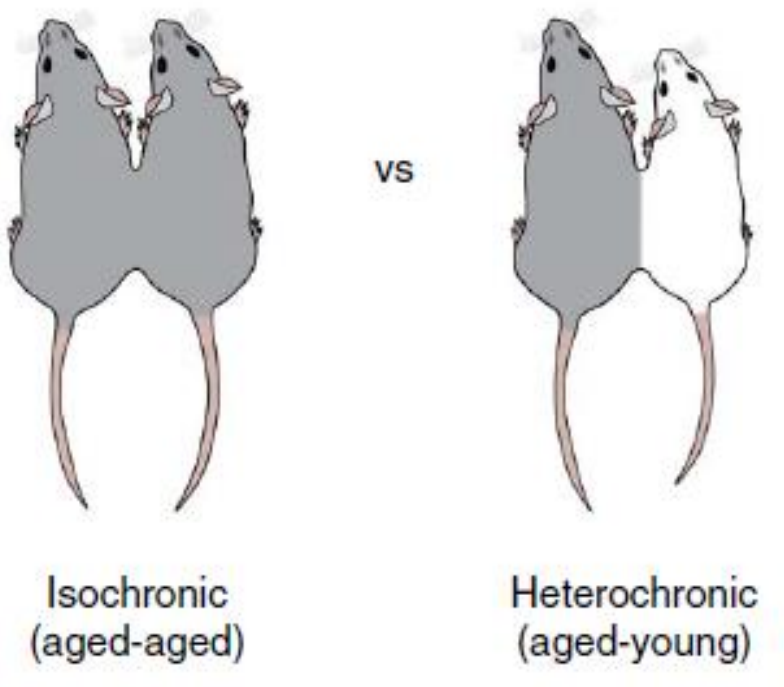
Can heterochronic parabiosis improve

- Molecular
- Structural
- Functional
- Cognitive

declines in brains of aged mice ?

Heterochronic parabiosis of aged (18mo) with young (3mo) mice

Isochronic parabiosis of aged with aged mice

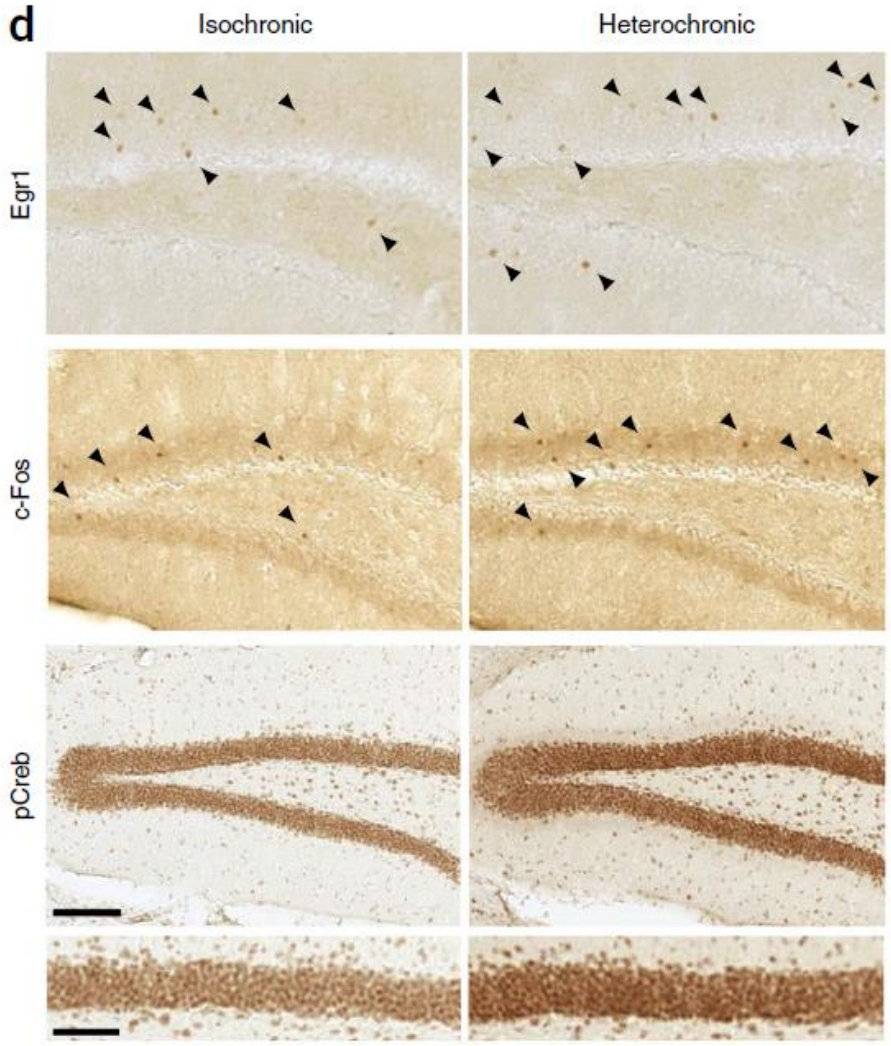


Results

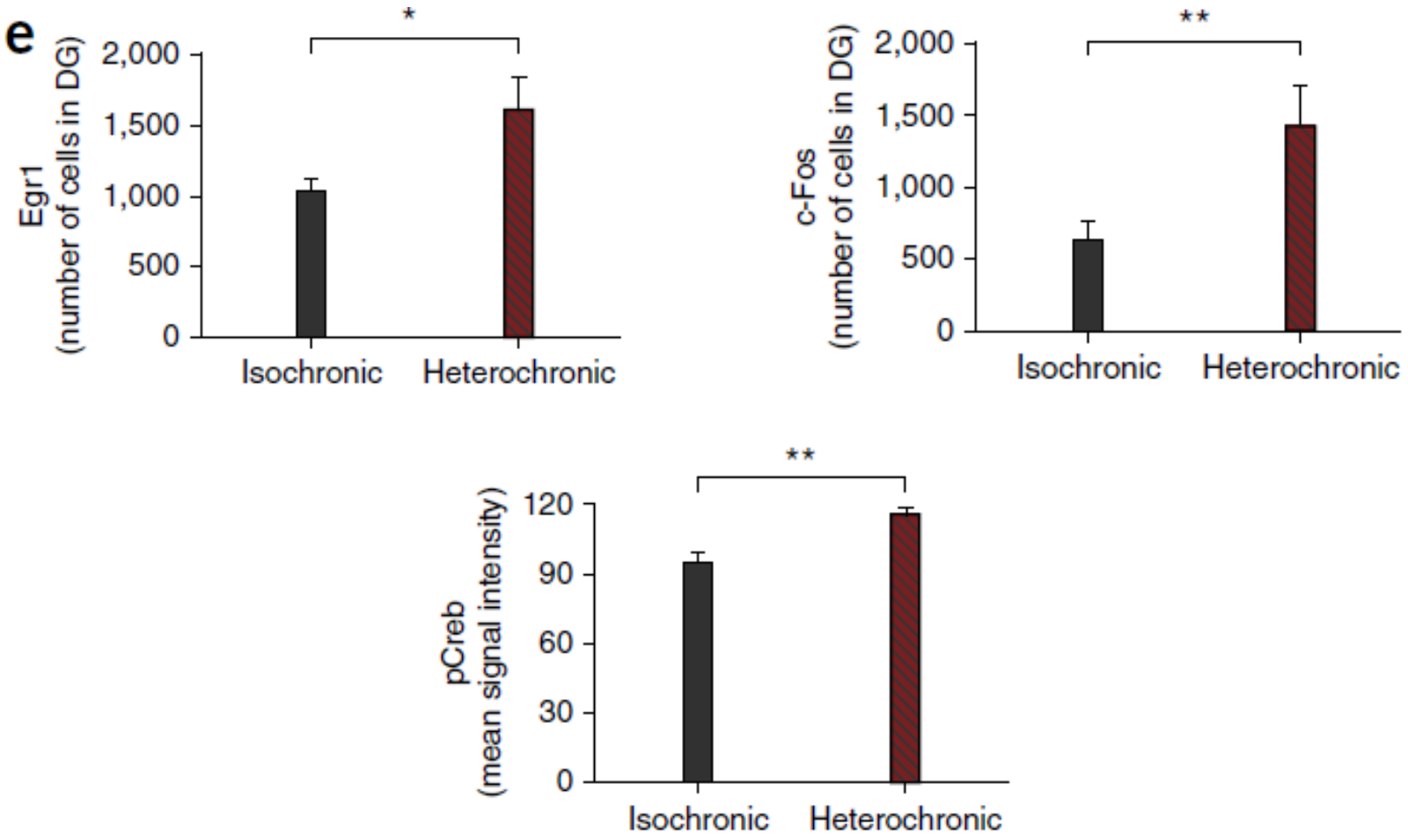
- Differences in synaptic plasticity regulating genes e.g. Creb
- Transcriptional profile indicates plasticity change in heterochronic parabionts
- Increased numbers of cells expressing immediate early genes
- Including EGR1 & c-fos
- And increase in phosphorylated Creb in DG and CA1

- Different immediate early gene expression and creb phosphorylation between young and aged unpaired animals
- No molecular changes

Increased numbers of cells expressing immediate early genes e.g. EGR1, c-fos & phosphorylated Creb in DG



Quantification of EGR1, c-fos & pCreb levels



Results

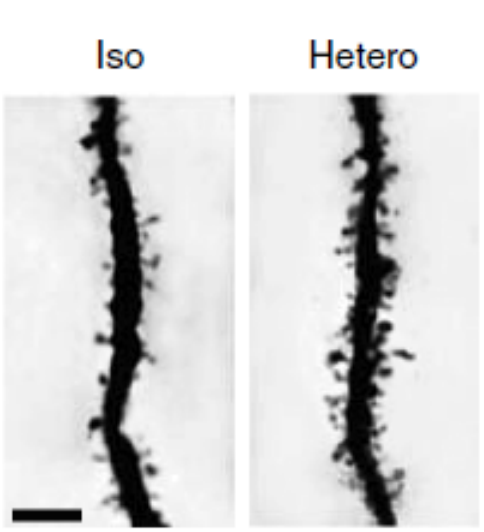
- Synaptic plasticity increased in aged hippocampus upon young blood

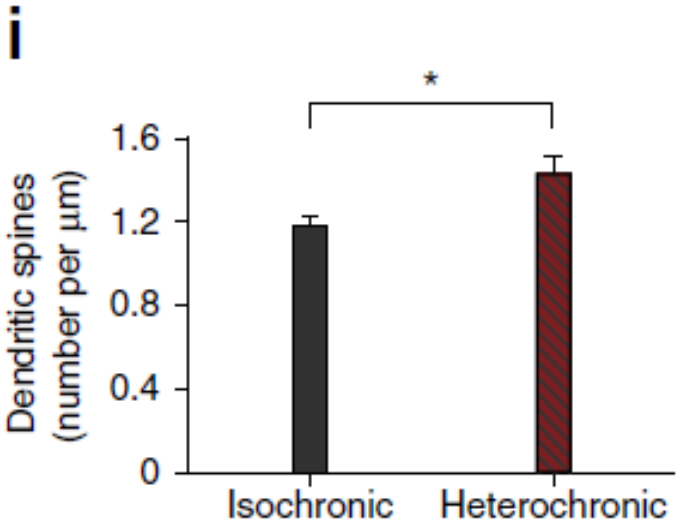
- Golgi analysis to check synaptic plasticity
- Dendritic spine number on granule cell neurons in DG increased thorugh young environment
- No difference in dendritic complexity

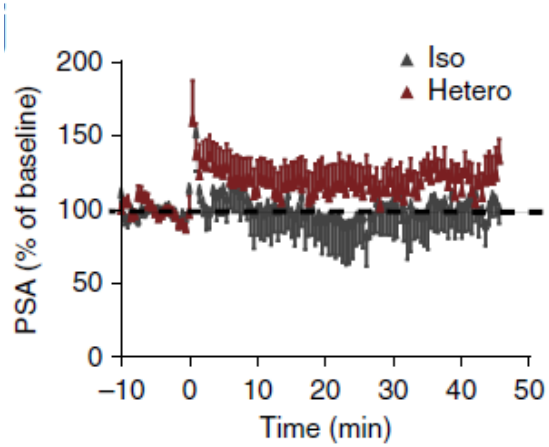
- Young blood increases spine number

- Extracellular electrophysiological recordings on hippocampal slices: LTP of heterochronic parabionts increased time above baseline

Changes in synaptic plasticity and after LTP



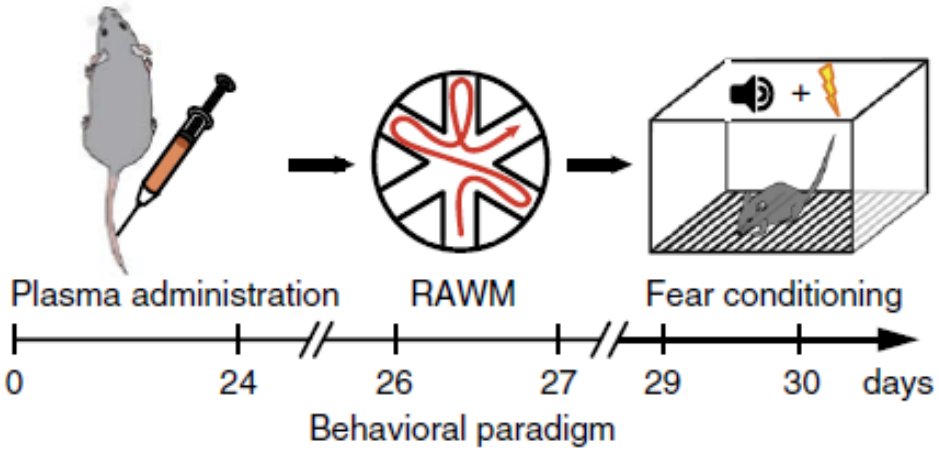


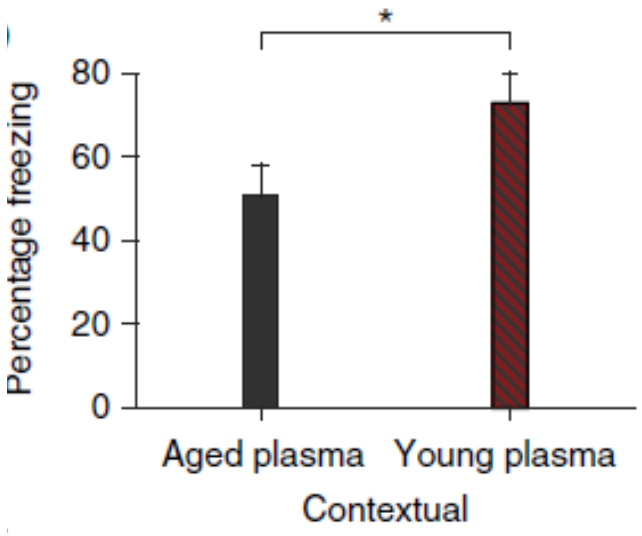


Results

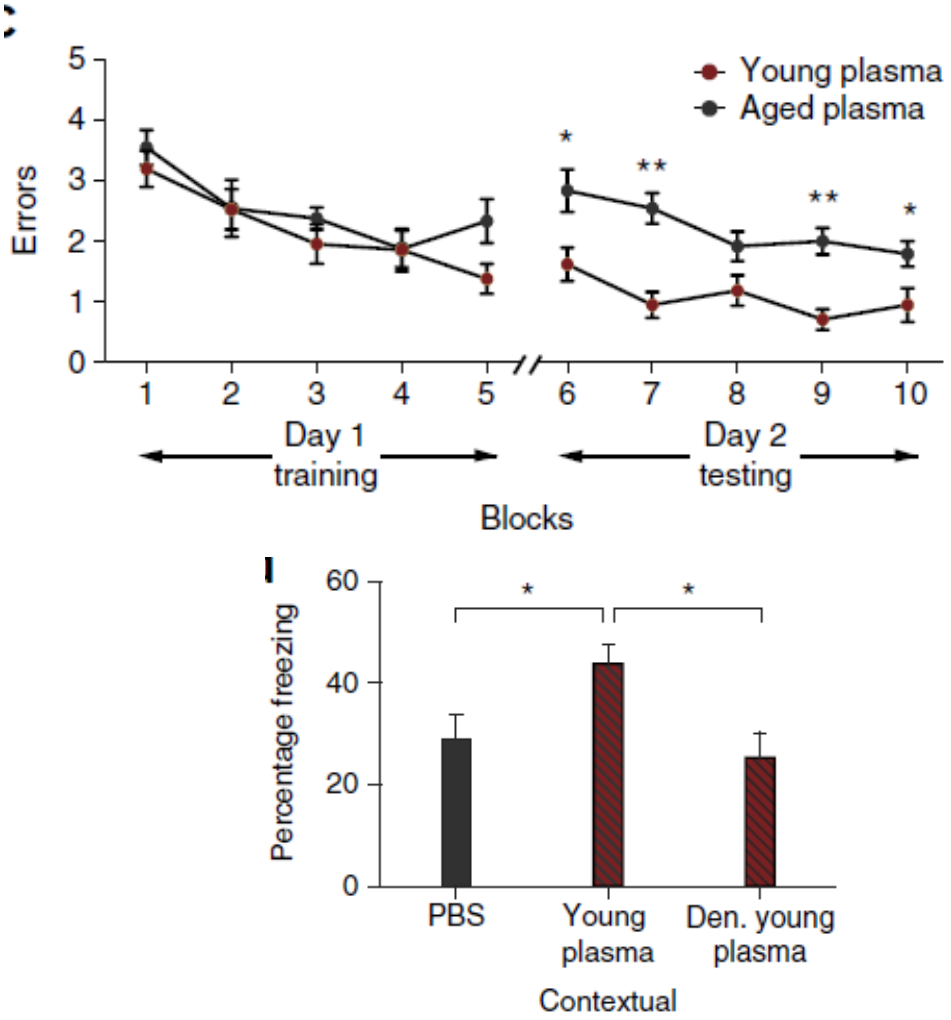
- RAWM with 100uL young or old blood plasma i.v.
- Fear conditioning: all mice similar baseline freezing
- With young plasma increased freezing in contextual but not cued memory testing
- young plasma treated aged mice showed enhanced learning and memory
- No differences between aged mice and aged mice with aged plasma
- Denaturated plasma decreased beneficial effect

Contextual fear conditioning after plasma treatment





RAWM & contextual fear conditioning 24 h after training

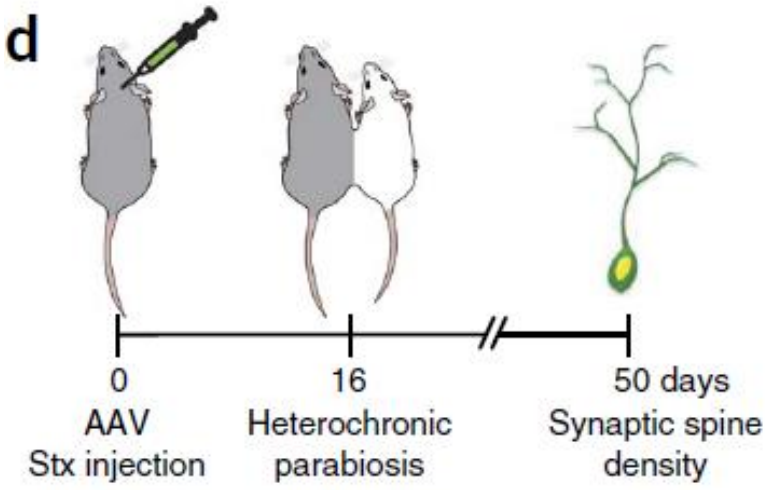


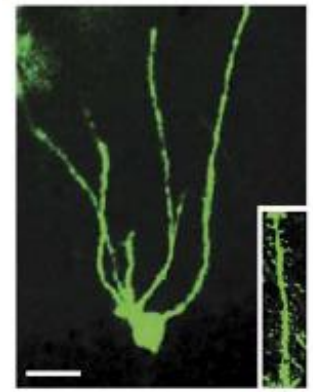
Results

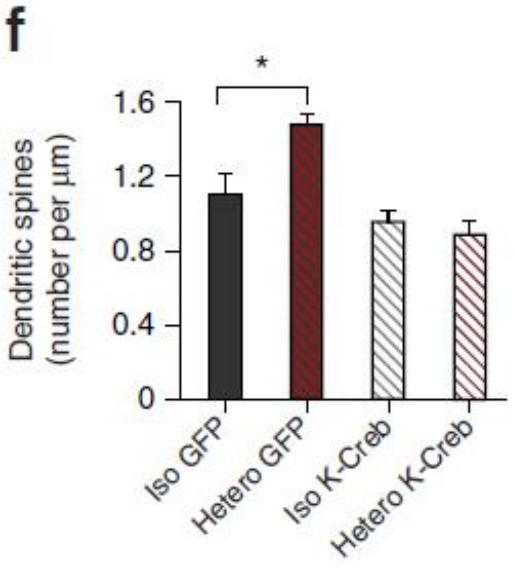
- Inhibition of Creb signaling in hippocampi of aged animals (AAV to inhibit Creb signaling)
- Injected into contralateral hippocampi

- Parabiosis after 2 weeks
- Beneficial effects on spines mitigated after Creb silencing
- Creb signaling is neccessary for spine number increase through young plasma

Results

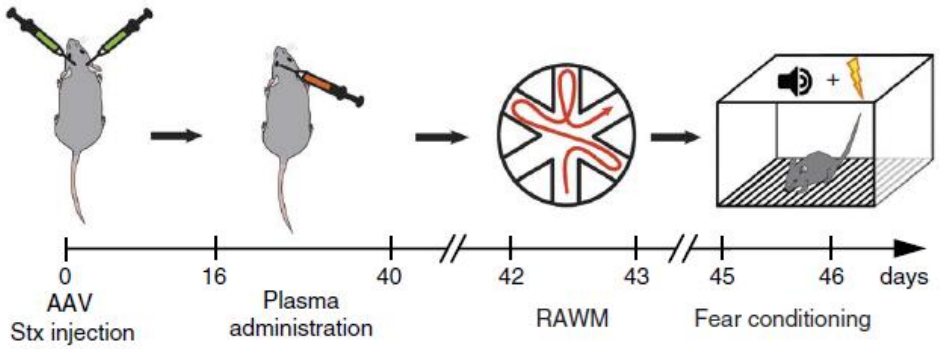

d
0
AAV
Stx injection
16
Heterochronic
parabiosis
50 days
Synaptic spine
density




f
*
Dendritic spines
(number per µm)
1.6
1.2
0.8
0.4
0
Iso GFP
Hetero GFP
Iso K-Creb
Hetero K-Creb

Results

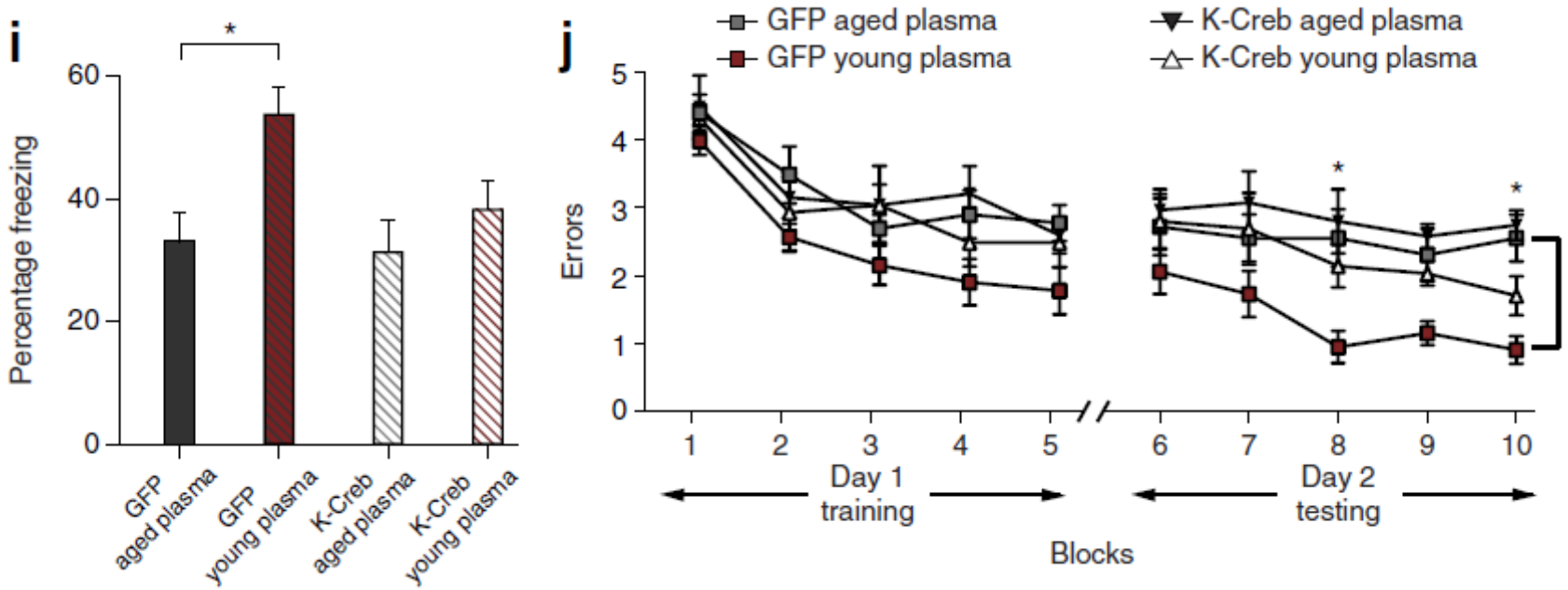
- Bilateral AAV injection
- Intraorbital plasma treatment (8x in 24d)
- behavior testing (RAWM, fear conditioning)



Results

- RAWM all groups smilar spatial learning capacity
- Similar swim speeds
- GFP young plasma had significant better leraning
- CREB mediated effect of young plasma in learning /memory

Contextual fear conditioning and RAWM



Conclusion

- Young plasma is rejuvenating synaptic plasticity
- Young plasma improves cognition of aged mice
- Reverse effects of brain ageing
- «pro-aging» factors were unaltered through the experiment
- Pro youthful factors from young blood reverse age related impairments

- Effect on aged humans?
- Effect on aged humans with neurodegenerative diseases?

JAMA Neurology | Original Investigation

Preclinical Assessment of Young Blood Plasma for Alzheimer Disease

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Key Points

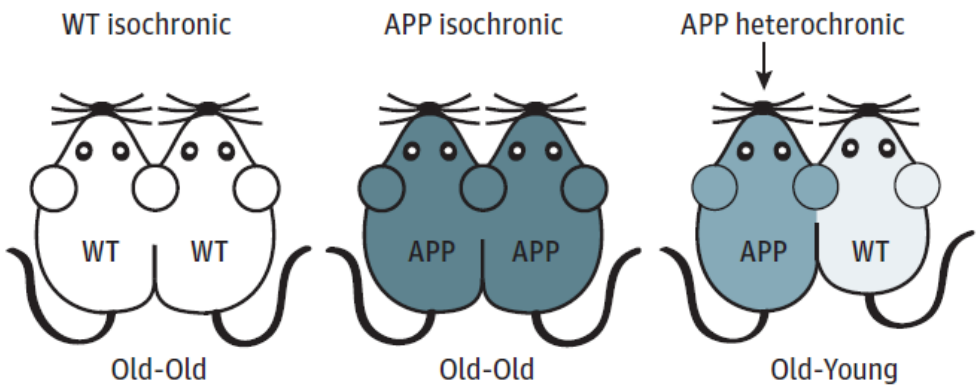
Question What is the effect of young blood factors on Alzheimer-like disease in mice?

Findings A mouse model of Alzheimer disease was exposed to young blood either through surgically facilitated shared blood circulation or intravenous plasma administration, which was associated with a partial restoration of molecular and behavioral disease-related deficits.

Meaning Young blood plasma benefits mice that model Alzheimer disease and could be a new therapy for humans.

aim

- AD pathology starts long before clinical symptoms manifest
- No way to prevent or delay the disease yet
- May young plasma ameliorates pathology and cognition in AD mice?
- Parabiosis or repeated injections of young (2-3 mo) plasma benefits old mice
- hAPP mice (FAD) with typical AD hallmarks (amyloid plaques, loss of synaptic and neuronal proteins, behavioral deficits) as old individuals
- Improvements in synaptic function and behavior observable
- Translation to clinics possible?

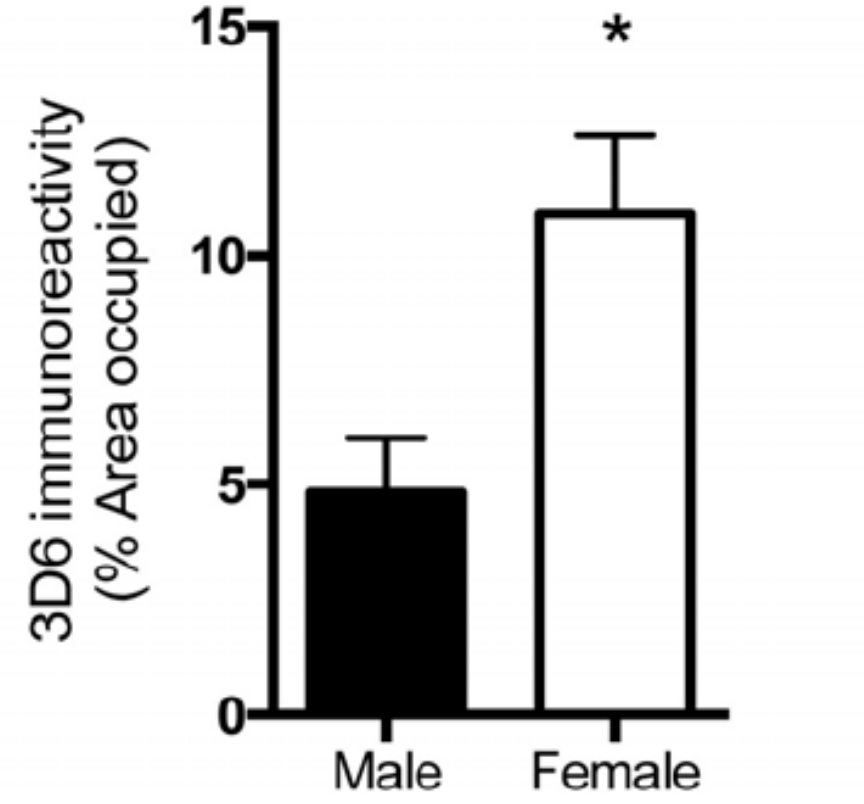


Setup

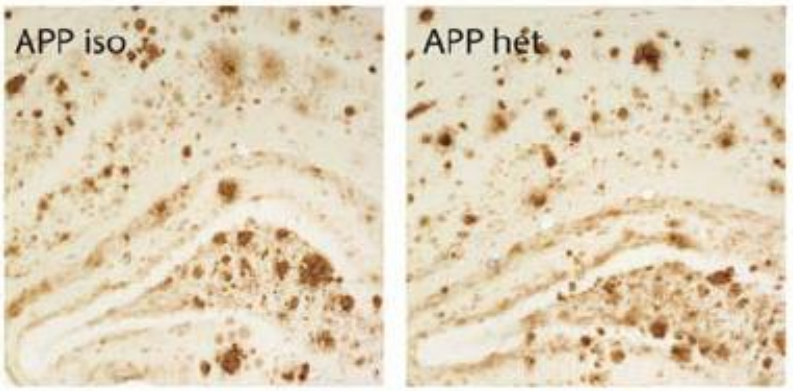
- Parabiosis
- Systemic treatment with pooled mouse plasma (n=120; 2-3mo) 2x/week; 4 weeks

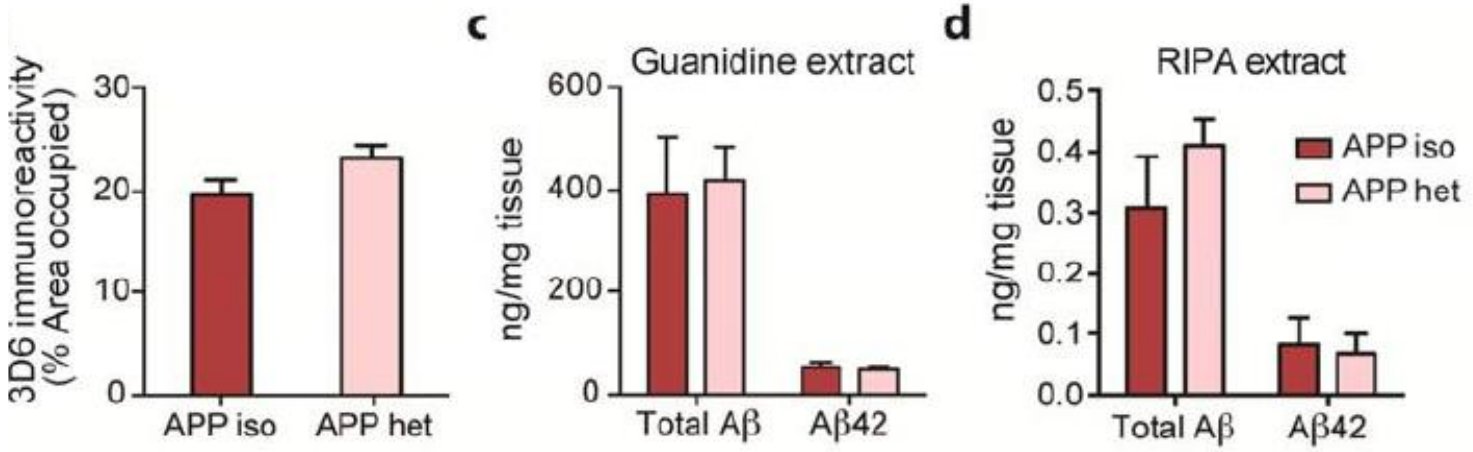
- RNA analysis (n=7/group)
- Y-maze
- Fear conditioning
- Smart homecage
- IHC

Total plaque amount

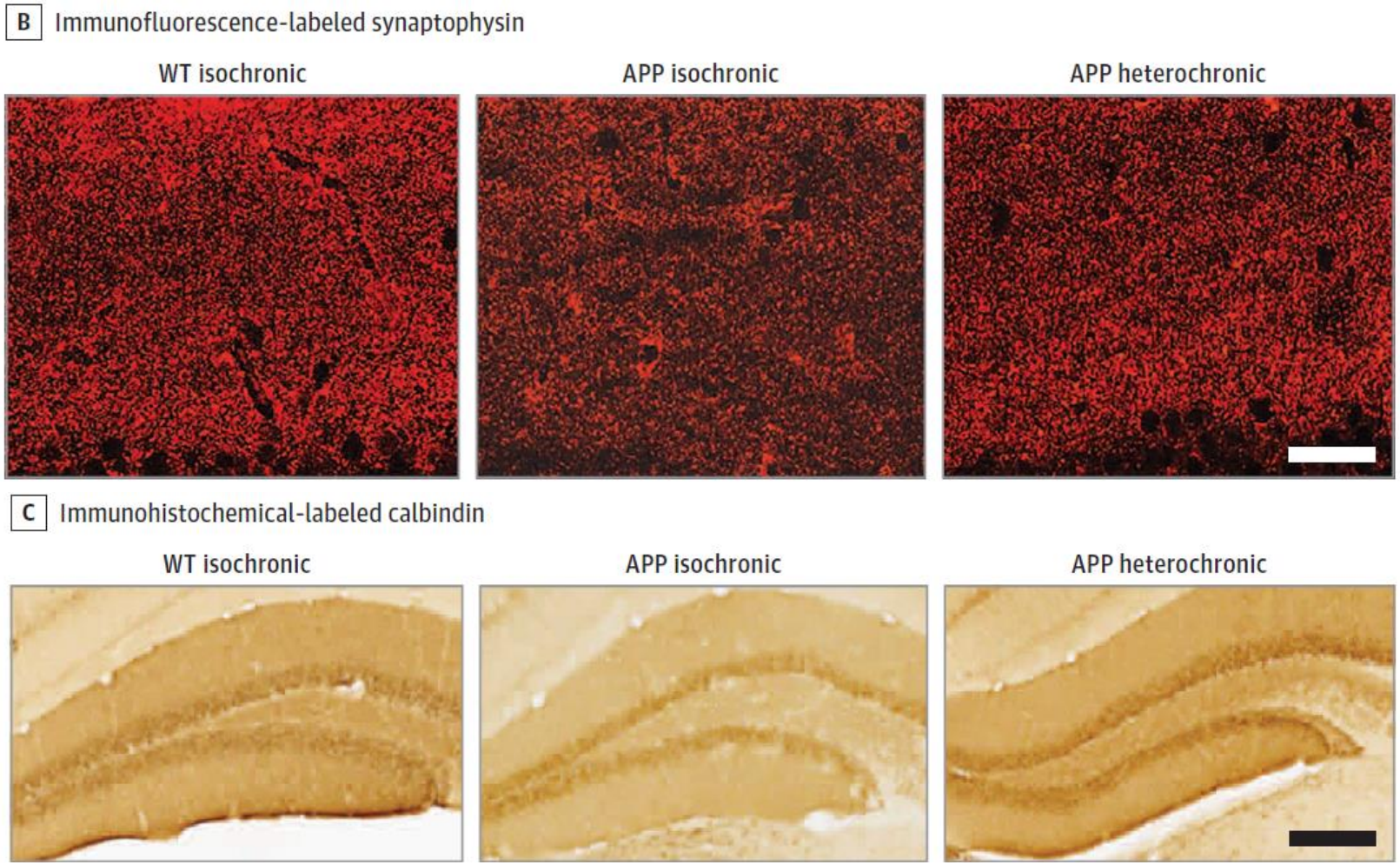


Heterochronic parabiosis has no effect on hippocampal Abeta levels

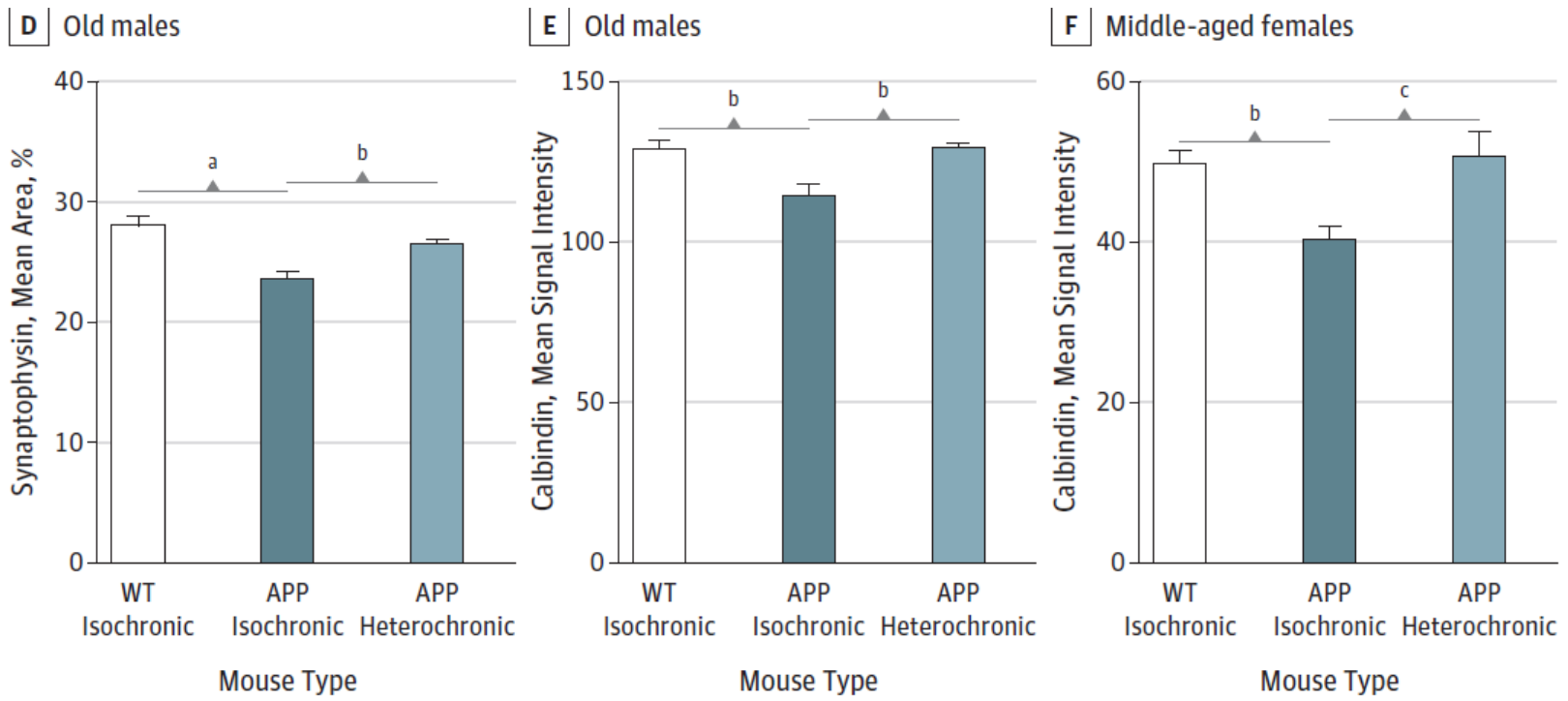




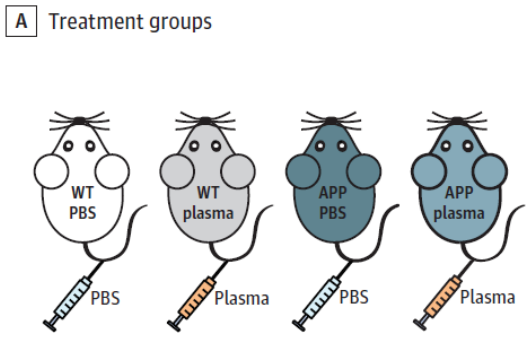
Parabiosis led to:
nearly complete restoration in levels of synaptic and neuronal proteins

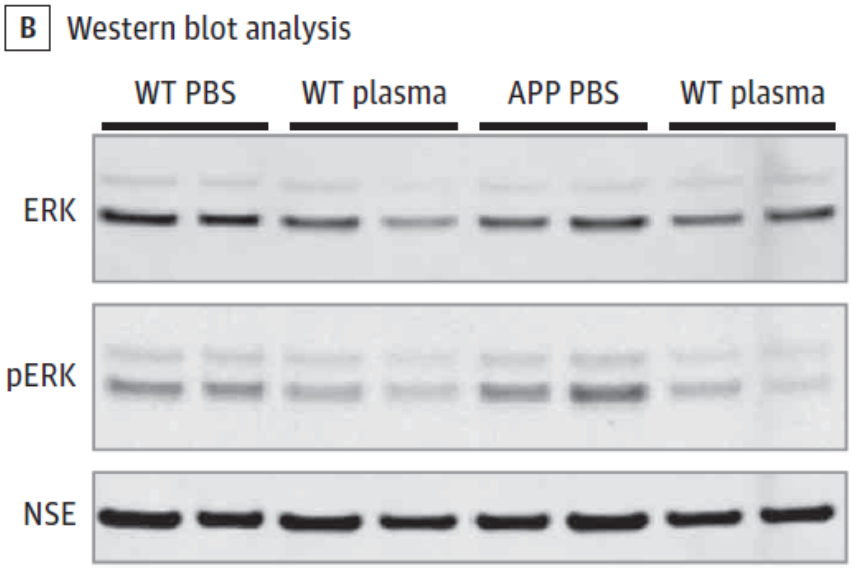


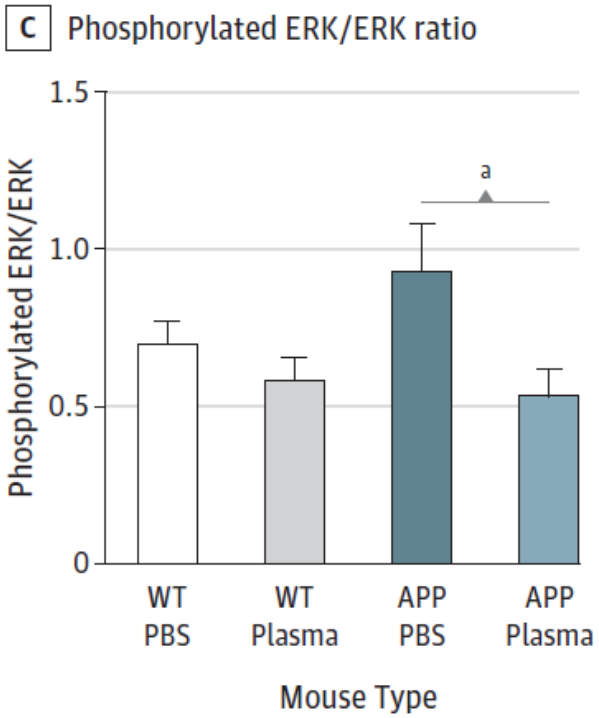
Quantification of synaptophysin and calbindin levels after parabiosis



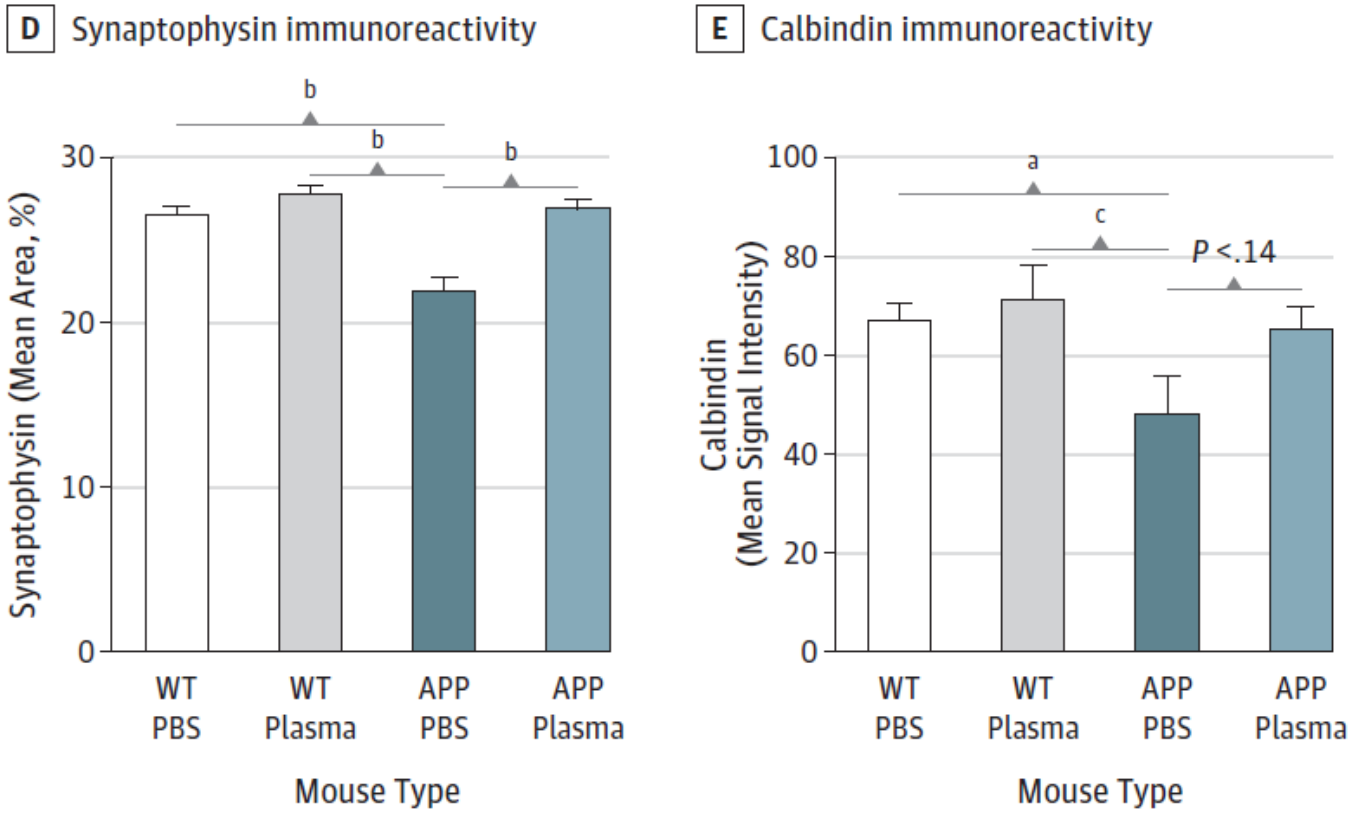
Effect of young blood plasma on memory of APP mice: lowered abnormal ERK activation



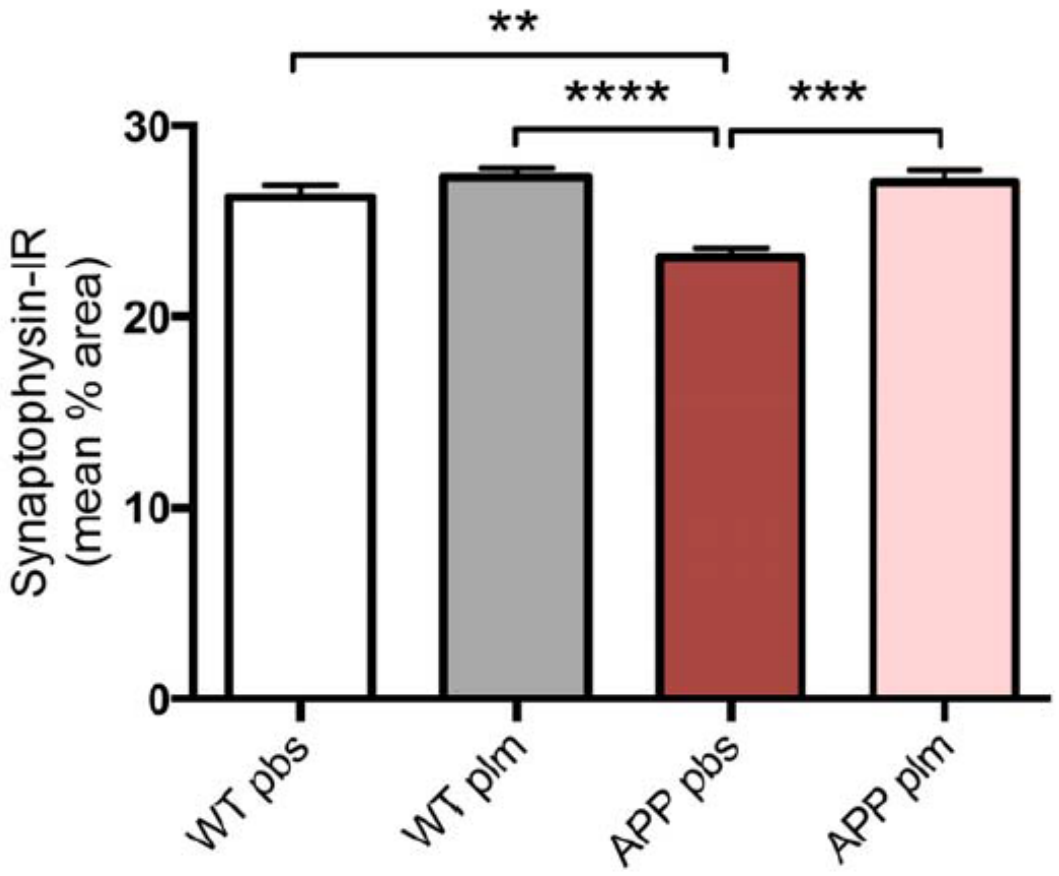




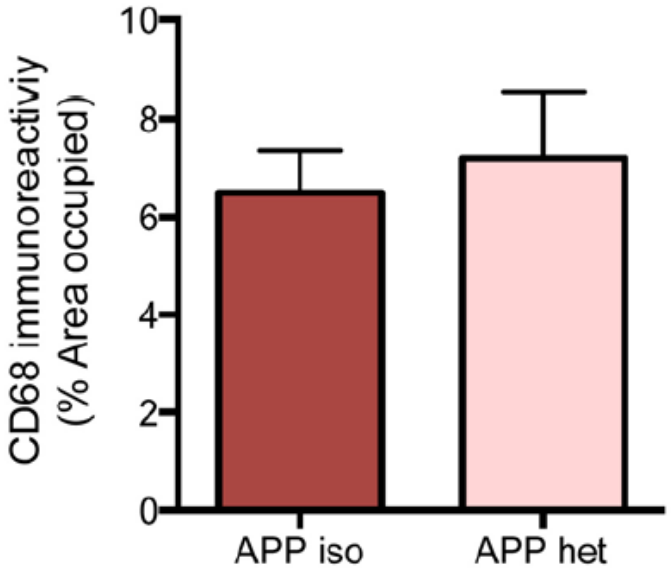
Restored synaptophysin & calbindin levels

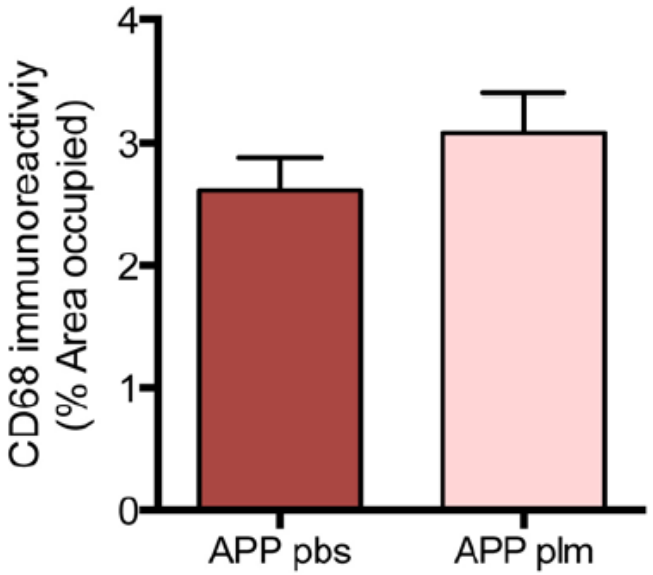


Young plasma restored synaptophysin depletion in mouse neocortex

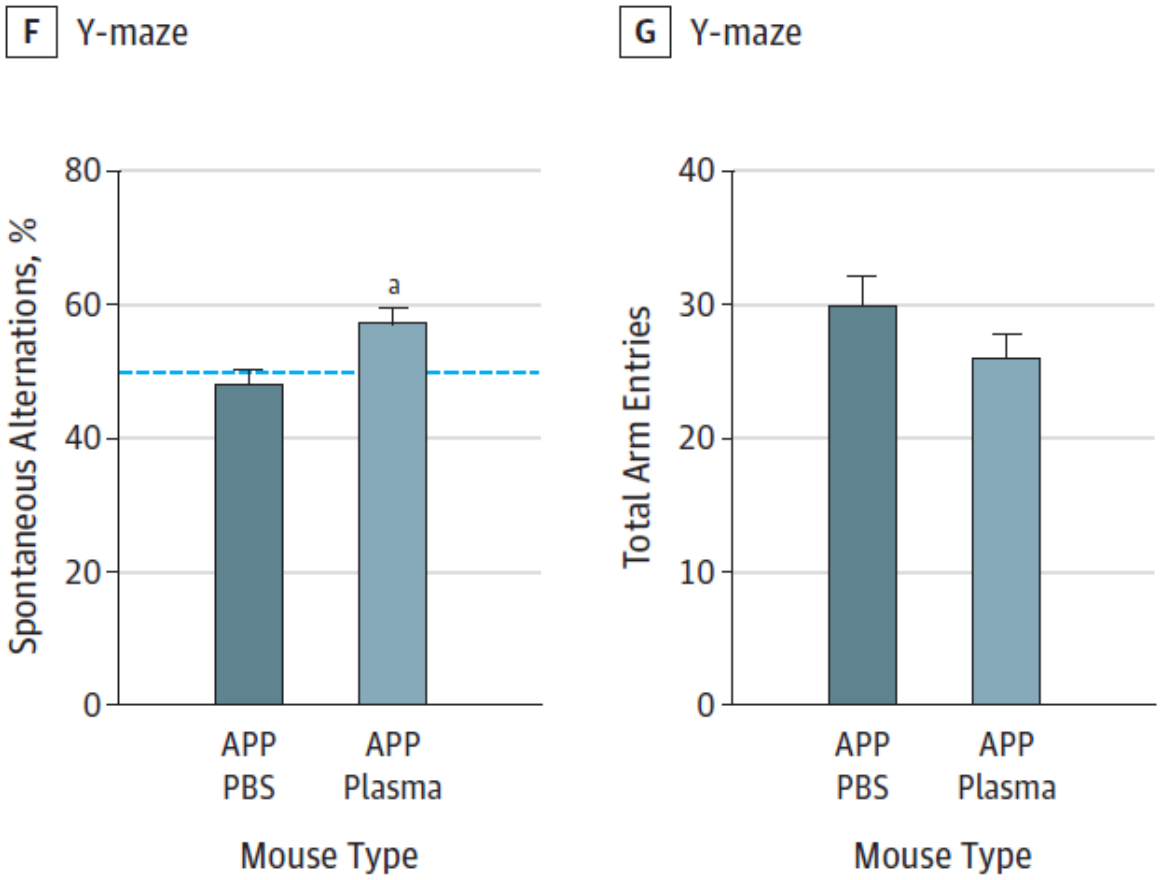


Heterochronic parabiosis and plasma administration has no effect on CD68^{+} area

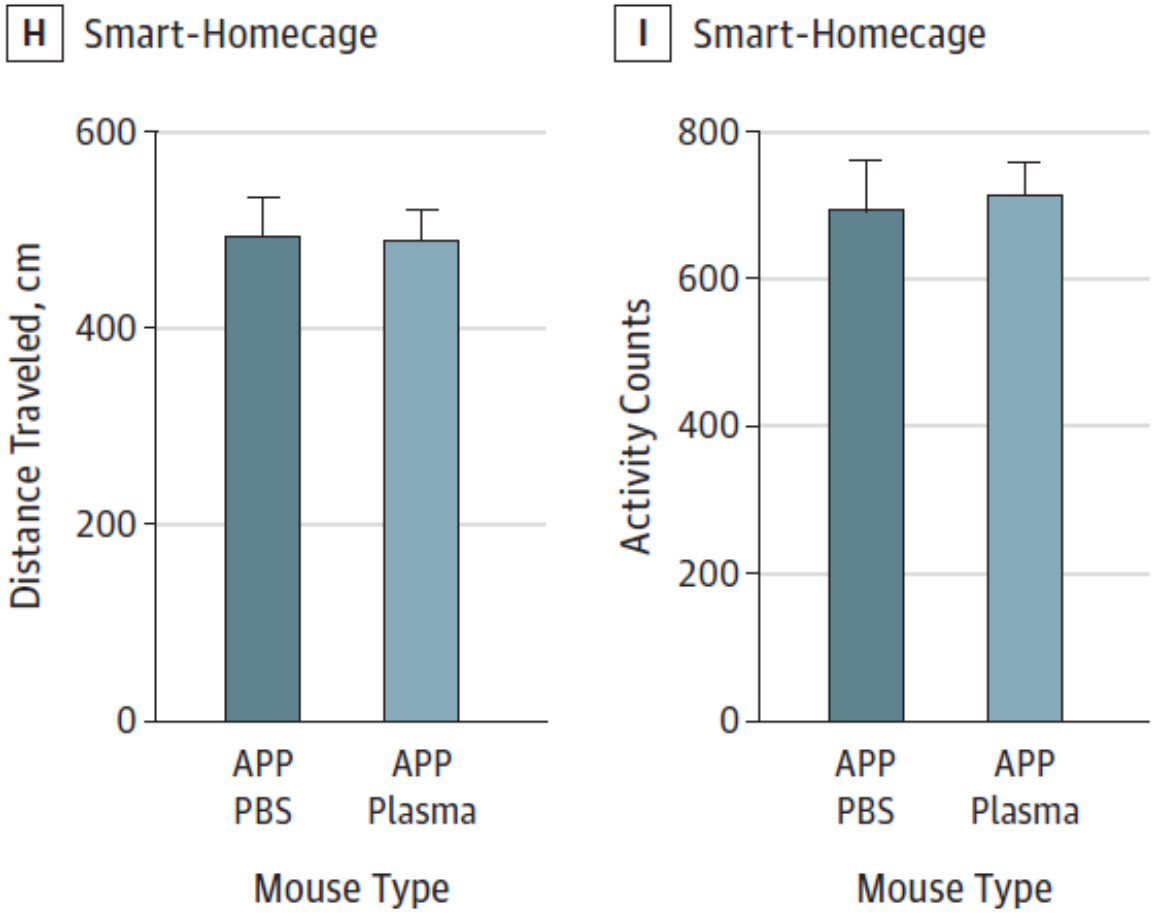




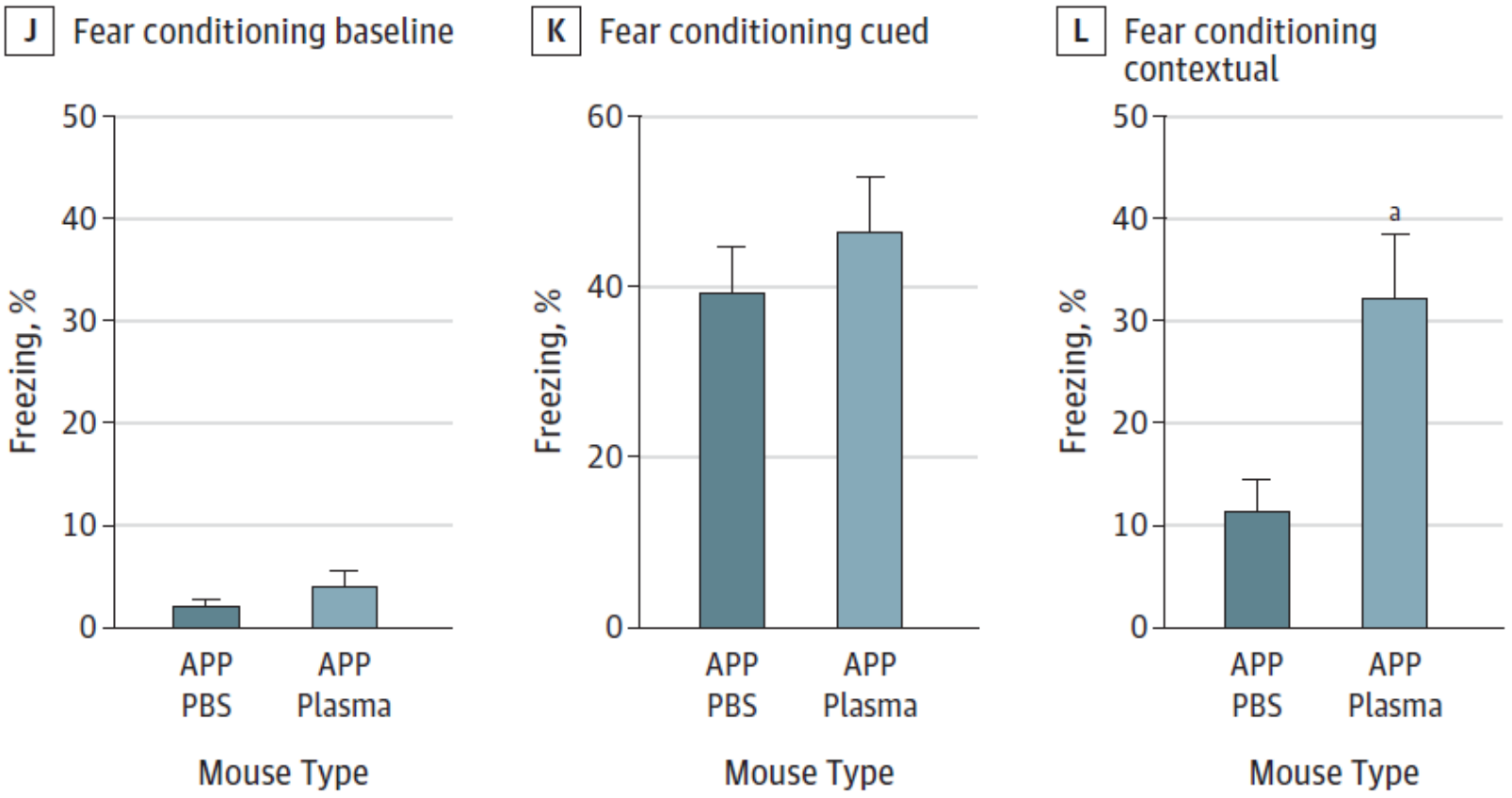
Young plasma treatment led to: Restored memory deficiency



Unaltered activity



More freezing upon contextual fear conditioning



Summary

- APP mutants showed reduction of synaptophysin and calbindin plus abnormal ERK activation – recovery through 5 weeks parabiosis/plasma
- Middle aged heterochronic females revealed same output – no sex specific effect
- Heterochronic parabiosis modulates key signaling pathway regulating genes in APP mice
- Memory deficiency reported in APP mice improved through young plasma
- No differences between groups in smart home cage (3h observation)

THANK YOU