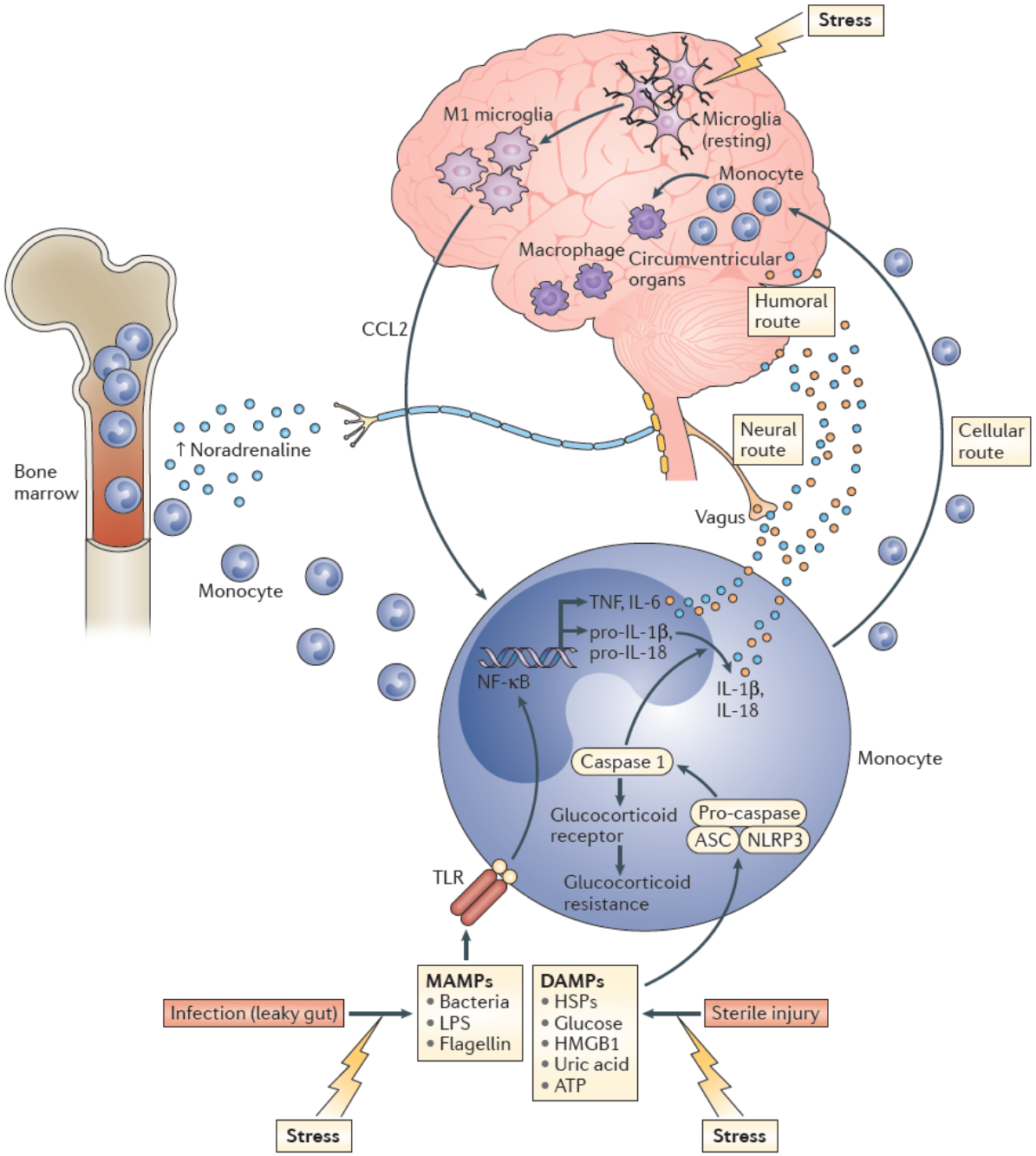
Lymphocytes from Chronically Stressed Mice Confer Antidepressant-Like Effects to Naive Mice

Brachman RA, Lehmann ML, Maric D, Herkenham M. Lymphocytes from Chronically Stressed Mice Confer Antidepressant-Like Effects to Naive Mice. The Journal of Neuroscience. 2015;35(4):1530.

Vorgestellt im JC-Applied Immunology: Daniel Bormann

Context of the study:

On the bidirectional communication between immune cells and the CNS



Transmission of inflammatory signals to the brain:

Putative pathways:

Humoral: "Leaky" BBB, and circumventricular organs as entry points of cytokines

Neural: Binding to afferent vagus fibers -> Induction of central cytokine secretion; Stimulation of ascending sympathetic fibers -> more catecholamine secretion -> vicious cycle.

Cellular: Trafficking of activated immune cells (typically monocytes) into vasculature and brain parenchyma, facilitated by activated microglia.

A focus on the cellular branch of the adaptive immune system

- Chronic psychological stress affects lymphocyte function, e.g. T-cell faciliated AB production by B-/Plasma Cells. (Possibly via an increment in stress hormone sensitivity) (Silberman et al. 2004)
- **Different subsets of lymphocytes confer specific effects on neural an hence behavioral parameters – Examples:**
  → Different methods of stress induction (e.g. restraint, learned helplessness increase Th17 cells in the CNS, conversly Th17 administration promotes depressive behavior, and blockage of Th17 activation rendered the mice resistant to learned helplessness. (Beurel et al. 2013)

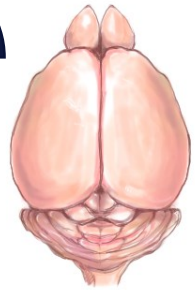
- Systemic depletion of CD4-positive T lymphocytes but not B- or CD8 T lymphocytes decreased learning, neurogenesis and CNS neurotrophic growth factor levels.

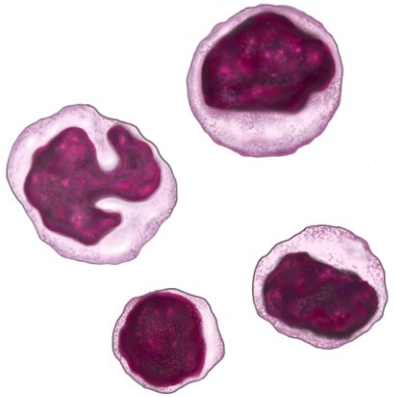
- Repopulation of RAG2-/-mice with CD4 lymphocytes fosters neurogenesis (Wolf et al., 2009).

Bottom line

CNS morphology
and function governs
the behavioral phenotype

Lymphocytes influence
CNS morphology and function





Higher-order
CNS function

Outside stimuli (e.g. chronic stress)
Influence Lymphocyte functioning

Environmental
parameters

Grossly simplified: Brachman et al. (2015) adressed whether the whole process can be described, full circle.

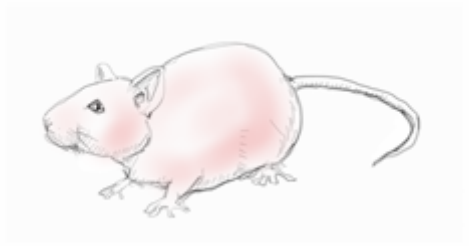
-> Does the psychological status alter lymphocyte function, which in turn alter the psychologcial status

Materials and Methods

Basic study design

Donor mice: WT male C57BL/6

Healthy Controls (HC)
regular group housing



Repeated Social
Defeat stress (SD)

After 14d: harvesting of lymphatic tissue,
extraction of MNCs

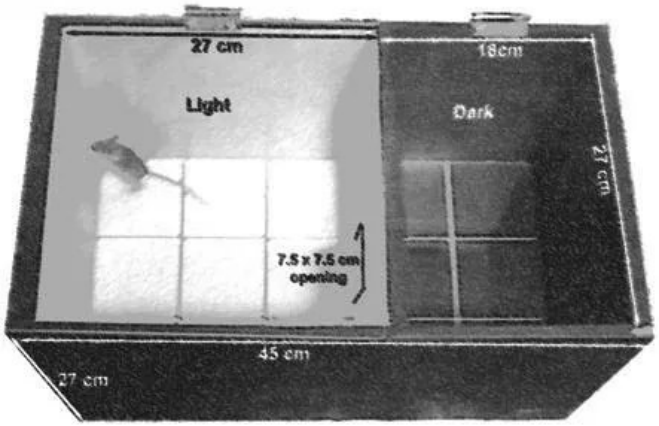
R.O. injection of 10-20x10e6 cells per host in 0.15ml sterile PBS, twice, with an 6d intervall into Rag2 -/- mice

3 groups:
HC -> Rag
SD -> Rag
Saline (SAL) -> Rag

Behavior experiments started 4 d and 10d after last transfer

Materials and Methods

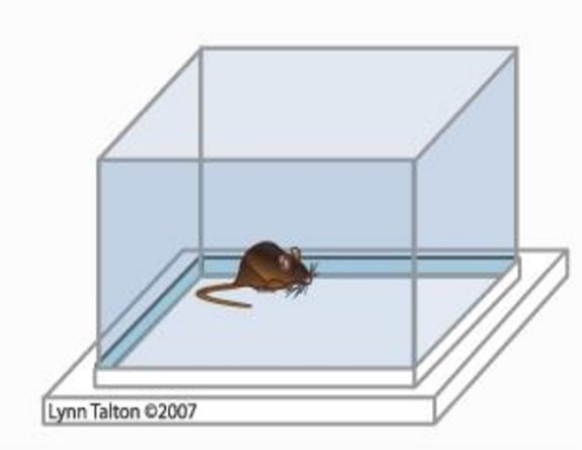
- **Behaviour: -> Measures of anxious behavior**



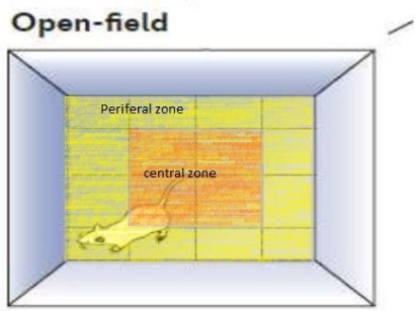
Light/dark box

(Transitions to light,

time spend in light)



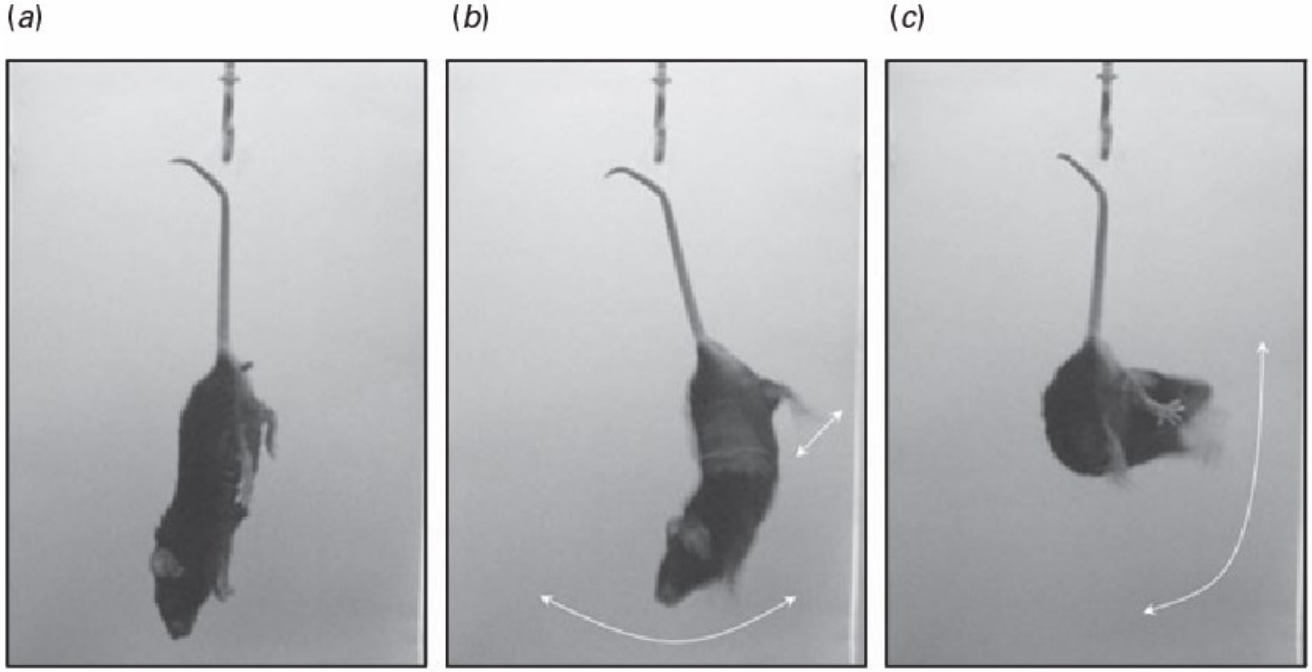
• **Open-field Test**



Open field test
(time spend in center)

https://i0.wp.com/www.matthewgthomas.co.uk/wp-content/uploads/2013/08/lightdarkbox.jpg

Immobility – depressive like behaviour Tail Suspension Test



All mice shown are rather displeased with the whole situation a.) is immobile, b.) and c.) activley try to disentagle themselves

https://www.semanticscholar.org/paper/Active-behaviours-produced-by-antidepressants-and-Berrocoso-Ikeda/bd1e051d4f6c5b949e8c77b89f754ee1e78e7623/figure/1

Materials and Methods

- **Measures of Social interaction**

→**Open field** -> How much time the animal will explore the area arround another dominant mouse

→**Urine scent-marking (USM):**
One corner of a blotting paper is spoted with urine from estrous female. How many urine marks will the male mice scatter across the whole blot and the area arround the female mark. (Mouse tinder date).

Materials and Methods

- **Flow cytometry:**

CD3-PerCP-Cy5.5, **CD4**-Alexa-Fluor 647, **CD8**-PE-Cy7, **CD25**-PE, and **CD19**-FITC

CD3: General T-Cell marker

CD4: Helper, **CD8:** Cytotoxic T-Cell marker
CD25: (= IL-2-R Alpha chain): Expressed on activated T-/B-/Treg cells

CD19: Expressed on most B-cells

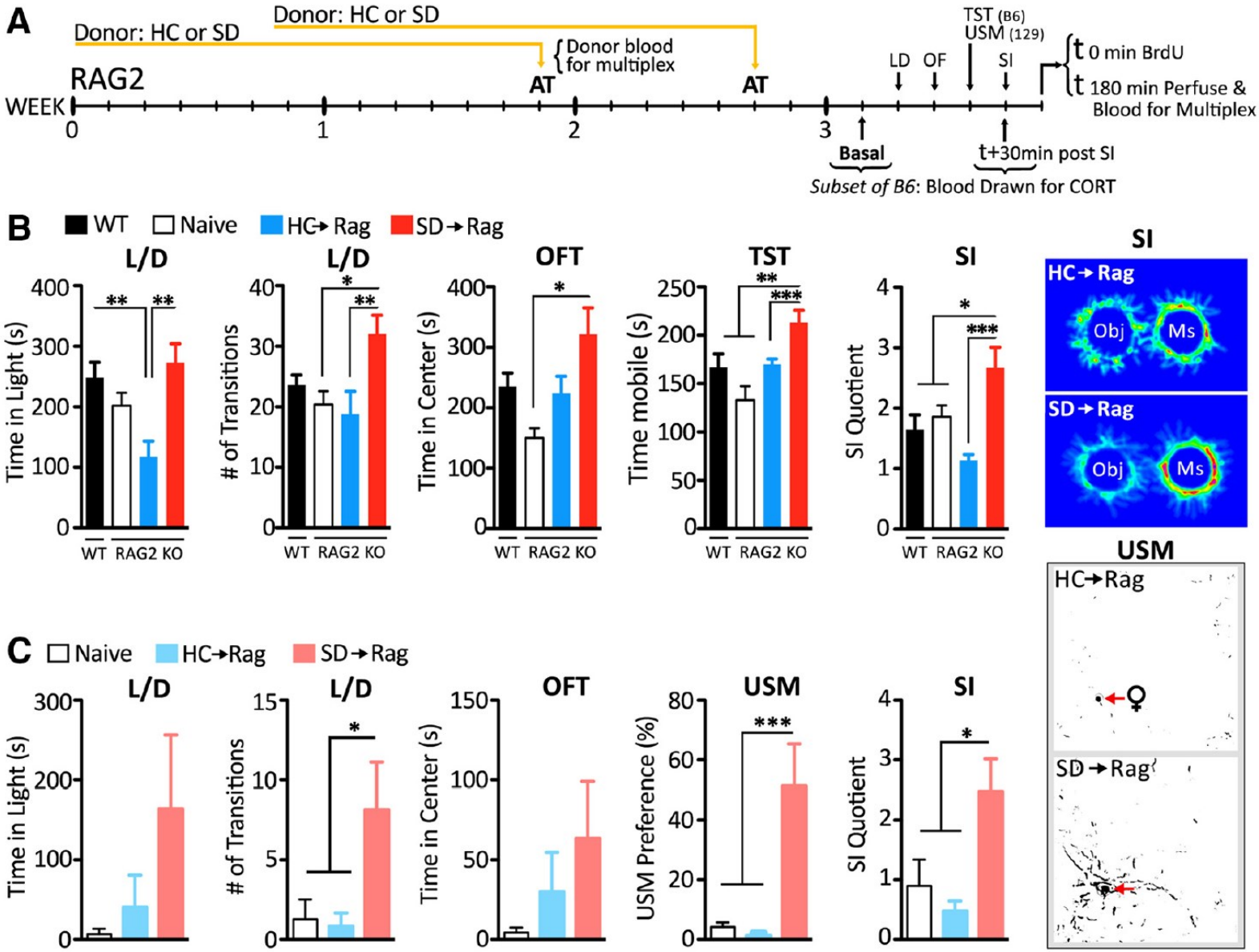
Separate Donor (HC and SD) mice were used .

For analysis of cell proliferation, donor cell suspensions from another set of mice treated as above were incubated in CFSE, injected in to Rag2 -/- mice, Recovered cells were CD3 marked.

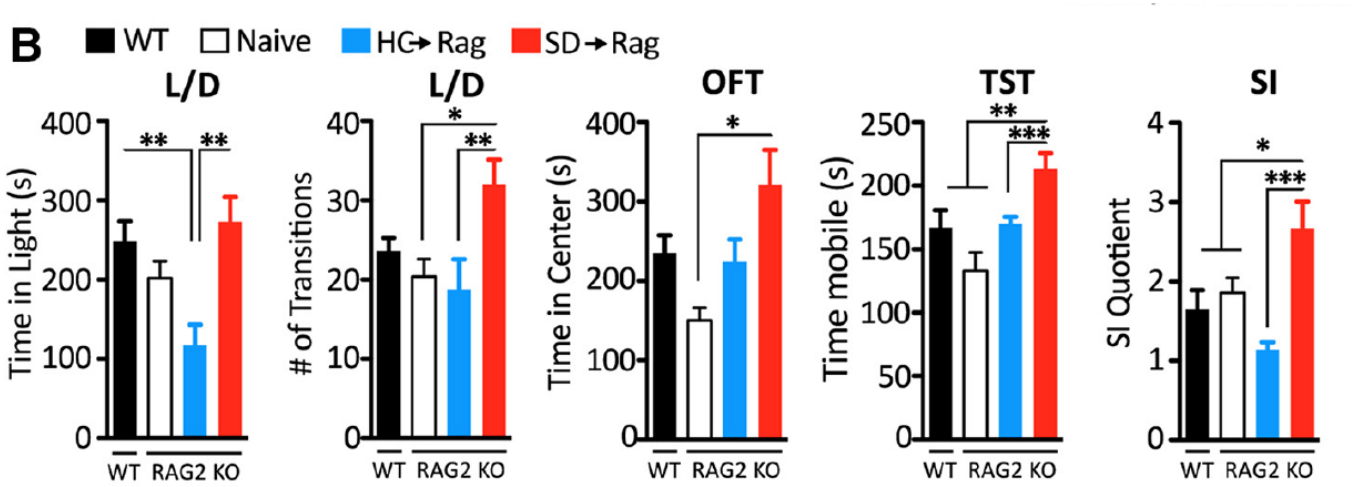
Materials and Methods

- **BrdU immunohistochemistry** was used to quantify hippocampal cell proliferation. (Rat anti-BrdU Abs + secondary biotinylated anti rat IgG, avitin-biotin-horse-radish peroxidase and DAB)
- **Corticosterone radioimmunoassays**, blood drawn from a set of mice after stressfull social interaction.
- **Serum protein determination** byMSQ-Plex Mouse Cytokine screen (Quansys) measuring IL-1b, IL-1a, IL-2, IL-3, IL-4, IL-5, IL-6, IL-10, IL-12p70, IL-17, MCP-1, IFNg, TNFa, MIP-1a, GMCSF, and RANTES.
- **Ex vivo microglia gene expression:** Assessment of number and viability of CD 11+ cells -> Stimulation of those cells with LPS, IL-4 -> Assessment of M1/2 polarization upon stimulation and **qRTPCR:** GAPDH, IL1-b, IL-6, MRC1

Results: Effects of lymphocyte transfer on behavior



Behavioral studys in Rag2 -/- mice with C57Bl/6 Background



Greater sociability compared to all other groups In SD->rag mice

HC->Rag mice spend least time in light, SD->rag most

SD>rag mice made Significantly more Transitions than the Other groups

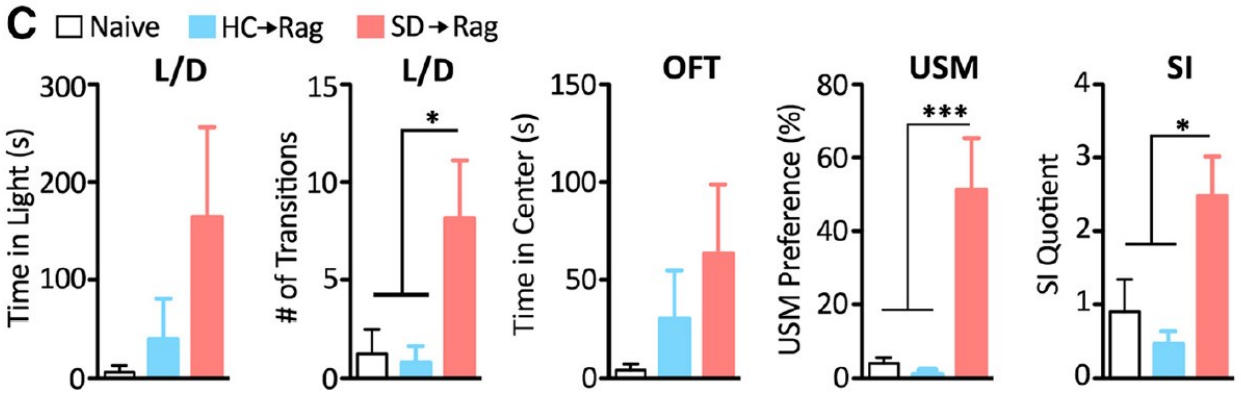
Significantly more time Spend in the OFT center SD->Rag vs. Naive

→ Anxiolytic Effect of SD-cell transfer?

Least time spend immobile Significantly less than in HC->Rag and Naive

→ Antidepressive Effect?

The behaviroal effects in 129 background mice mirrored the data from the C57BL/6 trial and where qualitative in nature

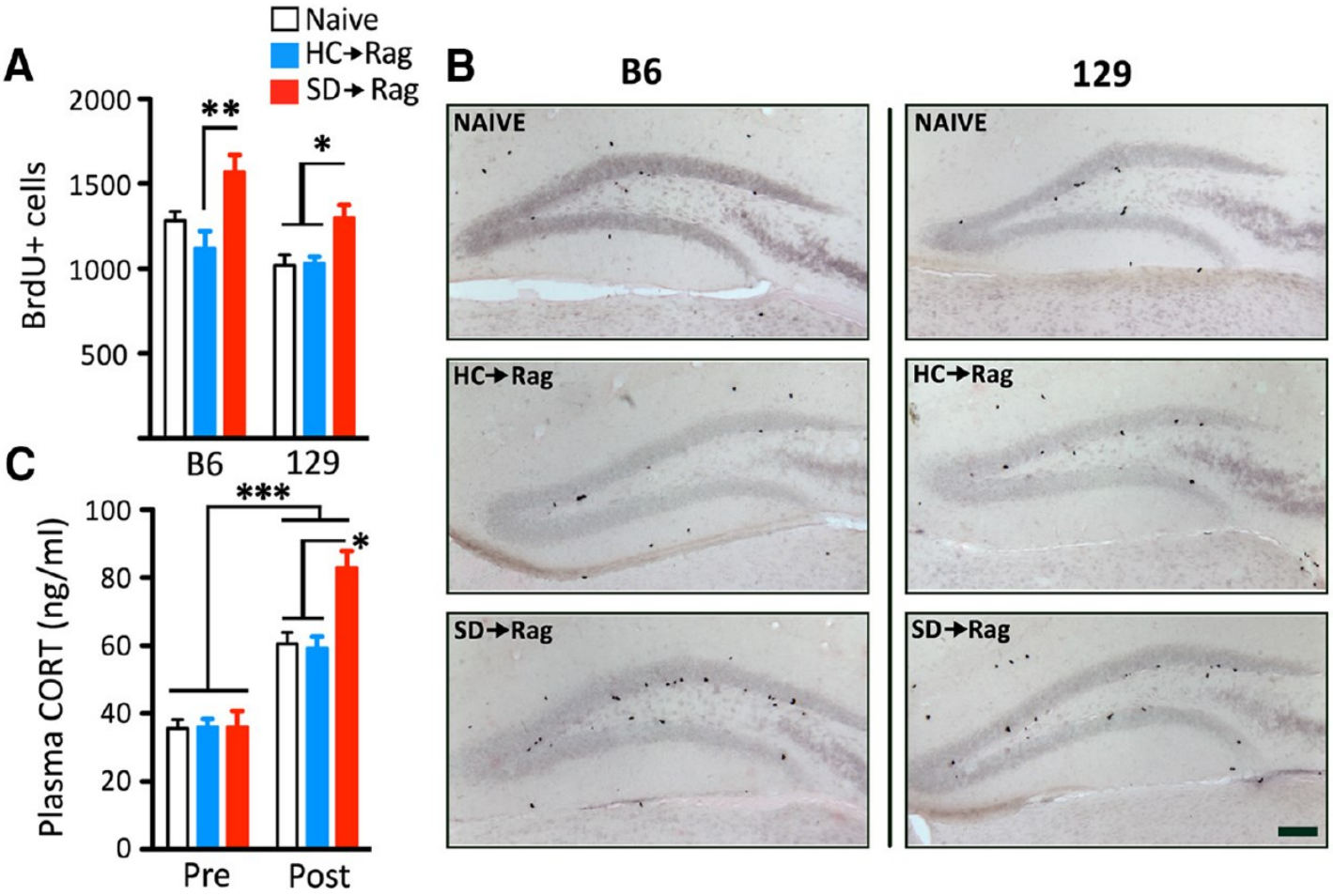


129 mice are known to be the least active mouse strain in OFT/L/D and sociability experiments!

SD->Rag 129 background mice behaved almost as C57BL/6 would!

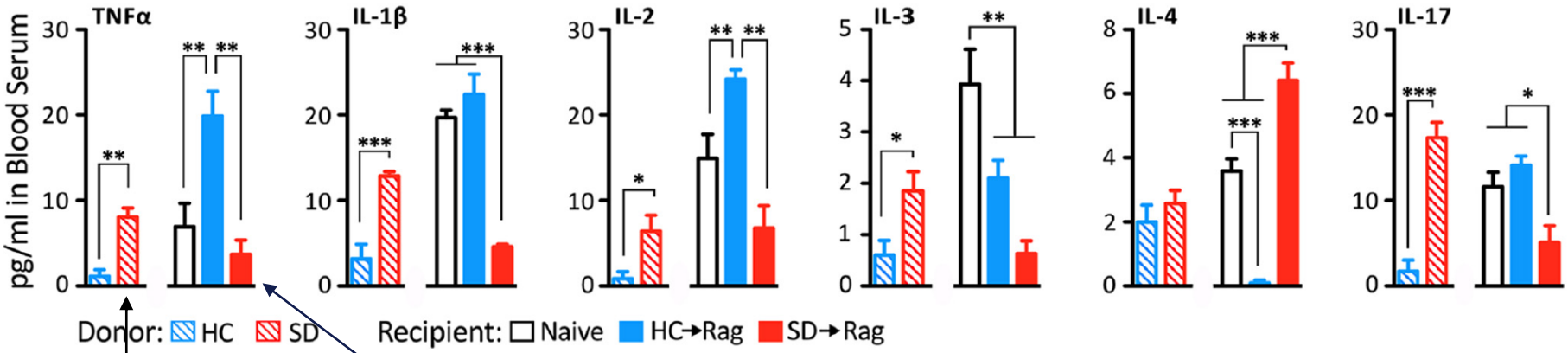
CAVE: Note the large error bars in the histogramms, large variability!

Transfer of lymphocytes from SD mice increased DG neuronal cell proliferation



Intrestingly corticosterone levels in the SD->Rag group were significantly higher than in the HC->Rag and Sal->Rag2-/- groups

Cytokine profiles in the blood of donor and recipient mice show opposite stress-induced and stressed cell-induced changes



Left column:
Donors

Right column
Lympocyte recipients
Rag2 -/-

Rag2-/- recipient mice levels of TNFa, IL-1b, IL-2, IL-3, and IL-17 were reduced in SD->Rag

Chronic defeat stress increased TNFa, IL-1b, IL-2, IL-3, IL-6, IL-17, and IFNg in the SD relative to HC groups

Microglial M1/M2 gene expression profiles are reversed between donor and recipient mice

Table 1. Microglial M1/M2 phenotype is differentially altered by chronic stress exposure in donor mice and by the source of stressed cells in Rag2 $^{-/-}$ recipient mice

Gene: treatment	HC	SD	HC→Rag	SD→Rag	Sal→Rag
IL-1β					
Media	1.10 ± 0.22	2.39 ± 0.28*	1.39 ± 0.46	0.78 ± 0.19	1.50 ± 0.06
IL-4	1.67 ± 0.23	1.51 ± 0.29	0.88 ± 0.17	0.92 ± 0.16	0.84 ± 0.13
LPS	7.15 ± 0.96***	20.4 ± 3.31***#	8.95 ± 0.80***	5.16 ± 0.93***	7.32 ± 1.62***
IL-6					
Media	1.06 ± 0.16	2.73 ± 0.26***	2.02 ± 0.27**	1.33 ± 0.16	1.74 ± 0.16*
IL-4	0.94 ± 0.15	1.92 ± 0.54	2.20 ± 0.41	1.64 ± 0.19	1.71 ± 0.17
LPS	6.20 ± 1.04***	13.7 ± 1.72***#	17.6 ± 2.14***#	7.82 ± 0.91***	23.8 ± 1.92***#
MRC1					
Media	1.03 ± 0.15	0.6 ± 0.08	0.54 ± 0.09***	1.14 ± 0.15	0.65 ± 0.05
IL-4	1.74 ± 0.39	1.61 ± 0.44	0.76 ± 0.12	1.41 ± 0.22	0.73 ± 014
LPS	0.83 ± 0.18	0.67 ± 0.12	0.36 ± 0.08***#	0.62 ± 0.13	0.64 ± 0.08
ARG1					
Media	1.04 ± 0.14	0.87 ± 0.25	0.56 ± 0.05	2.05 ± 0.22	1.16 ± 0.21
IL-4	22.0 ± 2.51***	9.13 ± 1.63***#	2.98 ± 0.44***#	4.81 ± 0.78***#	2.33 ± 0.41***#
LPS	1.33 ± 0.33	1.12 ± 0.17	1.12 ± 0.27	2.46 ± 0.96	1.23 ± 0.36

Values represent mean fold change ± SEM in gene expression compared to HC Media-stimulated samples ($n = 6$). Means with asterisk indicate significant difference versus HC media (*$p < 0.05$, **$p < 0.01$, ***$p < 0.005$). # indicates significance versus within-treatment HC sample ($p < 0.01$).

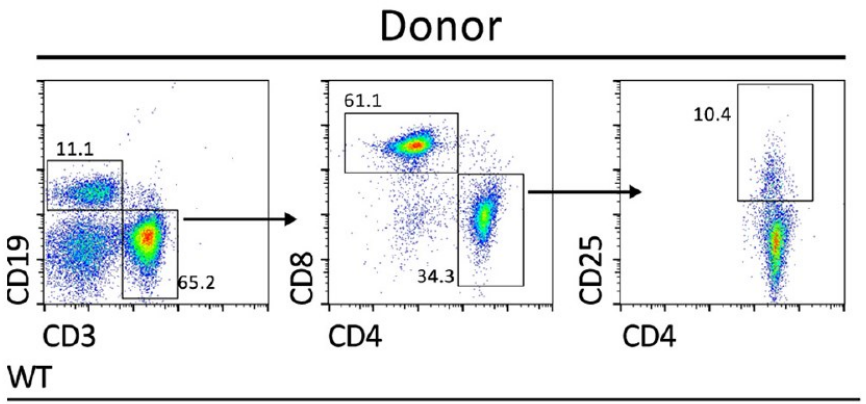
IL 1b, IL 6 = M1 markers

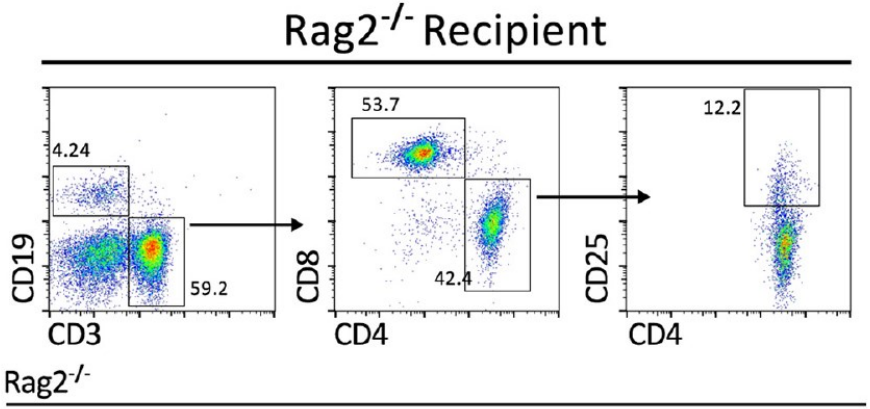
MRC1 ARG1 = M2 markers

Media: Basal state
IL-4 -> Stimulus for M2
LPS -> Stimulus für M1

SD donor microglia:
More polarized towards M1
Reversed in SD recipients!
M1 polarization in HC->Rag
M2 in SD->Rag

Lymphocyte subpopulations in the donor and recipient mice are not differentially affected by defeat stress





Group	% of Viable Cells		% of CD3⁺ Cells		% of CD4⁺ Cells
	CD3⁺	CD19⁺	CD4⁺	CD8⁺	CD25⁺
HC	65.8 ± 3.7	13.9 ± 2.0	33.7 ± 0.9	62.1 ± 1.3	9.7 ± 0.4
SD	67.9 ± 2.8	13.8 ± 0.8	31.2 ± 1.1	64.3 ± 1.2	10.7 ± 0.9

Group	% of Viable Cells		% of CD3⁺ Cells		% of CD4⁺ Cells
	CD3⁺	CD19⁺	CD4⁺	CD8⁺	CD25⁺
HC→Rag	51.3 ± 1.7	5.9 ± 0.5	42.8 ± 0.7	50.3 ± 1.8	10.5 ± 0.7
SD→Rag	43.4 ± 4.2	7.9 ± 1.4	44.6 ± 1.9	50.5 ± 1.5	10.5 ± 1.0
Sal→Rag	0.3 ± 0.2	0.1 ± 0.1	0	0	0

- Donor SD and HC mice showed no differences in relative cell numbers in lymph nodes
- Saline-injected Rag2-/- mice had virtually no lymphocytes
- In SD->Rag and HC->Rag mice T-cells remained abundant but B cells dropped from to 6% of all lymphocytes in the Rag2-/- host
- CD4-CD8 ratios were comparable among donors and in the recipient groups

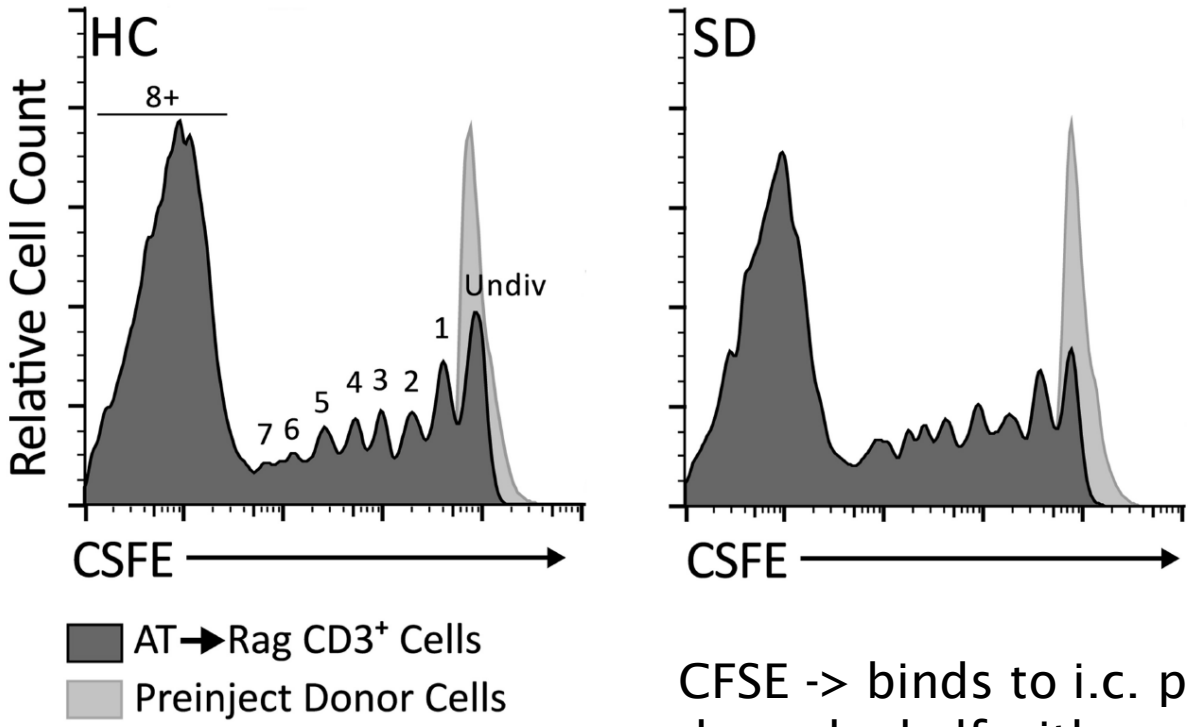
Table 2. Enumeration of lymphocyte subsets in spleen and blood of host Rag2 $^{-/-}$ mice

	% of viable cells		% of CD3 $^{+}$ cells		% of CD4 $^{+}$ cells
Group	CD3 $^{+}$	CD19 $^{+}$	CD4 $^{+}$	CD8 $^{+}$	CD25 $^{+}$
Spleen					
HC⟶Rag	11.1 ± 0.9	2.3 ± 0.3	36.3 ± 0.8	58.2 ± 1.4	6.1 ± 1.3
SD⟶Rag	11.4 ± 1.7	1.9 ± 0.4	36.9 ± 2.6	59.0 ± 2.5	7.3 ± 0.9
Sal⟶Rag[a]	0.3 ± 0.1	0.1 ± 0.1	0	0	0
Blood					
HC⟶Rag	18.6 ± 4.7	0.1 ± 0.0	46.1 ± 5.7	42.0 ± 8.6	0.7 ± 0.4
SD⟶Rag	21.4 ± 2.9	0.3 ± 0.1	47.1 ± 5.4	42.8 ± 5.3	0.9 ± 0.1
Sal⟶Rag[a]	0.3 ± 0.3	0.1 ± 0.1	0	0	0

[a]Lymphopenic mice had significantly less of each cell type compared to adoptive transfer groups ($p < 0.01$).

CD4+/CD8+ cell ratios in the host mice were different in different tissues but they were not different
in the stress versus unstressed conditions.
T-reg cells remained fairly constant

Lymphocyte proliferation is the same in HC->Rag and SD->Rag mice



CFSE -> binds to i.c. proteins, intensity drops by half with each cell divison -> peaks in the histogramm.
Most cells have underwend 8+ divisons.

Discussion

- Lymphocytes of the adaptive immune system are programmed by psychosocial stress in a way that allows them to confer anti-stress effects on the host. (Dramatic effect in 129 strain recipient mice).
- Intrestingly, lymphocytes from SD mice confered neurogenesis and elvated corticosterone levels. This contradicts the dogma of a general neurogenesis depressing effect of corticosterone → Context-Dependence of signaling?
- The blood cytokine and M1/2 polarizing effect of SD on lymphocytes was reversed in recipients.
- Previous studies used the adoptive transfer model to study the effect of different lymphocyte subpopulation with sometimes contradicting results.
- This was the first study to show that in vivo psychological manipulation changed the state of lymphocytes in a way that apparently compels them to adapt to a previously encountered stressor.

- The study confirms the hypothesis, that psychological stress might induce an immunological memory, that counterbalances disequilibrium, e.g. elicited by an ongoing inflammatory cascades upon disstress.
- **The study opened up several open questions:**

  → Precise molecular triggers, necessary and sufficient to elicit lymphocyte adpatation

  → Mechanism of long term adaption (e.g. Epigenetic changes?)

  → Relative role of the different molecular changes (e.g. neurogenesis, immunomodulation) on the confered behavioral effect and their interconnectedness

  → Mechanism of the immunomodulatory reversal effect (cytokine, microglia)

  → Short time span: How long could this effect possibly last?

  → Are peripheral lymphocytes a feasible target for the treatment of anxiety and depression

References

Beurel E, Harrington LE, Jope RS. Inflammatory T helper 17 cells promote depression-like behavior in mice. Biol Psychiatry. 2013;73(7):622-30.

Brachman RA, Lehmann ML, Maric D, Herkenham M. Lymphocytes from Chronically Stressed Mice Confer Antidepressant-Like Effects to Naive Mice. The Journal of Neuroscience. 2015;35(4):1530-8.

Miller AH, Raison CL. The role of inflammation in depression: from evolutionary imperative to modern treatment target. Nat Rev Immunol. 2016;16(1):22-34.

Silberman DM, Ayelli-Edgar V, Zorrilla-Zubilete M, Zieher LM, Genaro AM. Impaired T-cell dependent humoral response and its relationship with T lymphocyte sensitivity to stress hormones in a chronic mild stress model of depression. Brain Behav Immun. 2004;18(1):81-90.

Wolf SA, Steiner B, Akpinarli A, Kammertoens T, Nassenstein C, Braun A, et al. CD4-Positive T Lymphocytes Provide a Neuroimmunological Link in the Control of Adult Hippocampal Neurogenesis. The Journal of Immunology. 2009;182(7):3979.