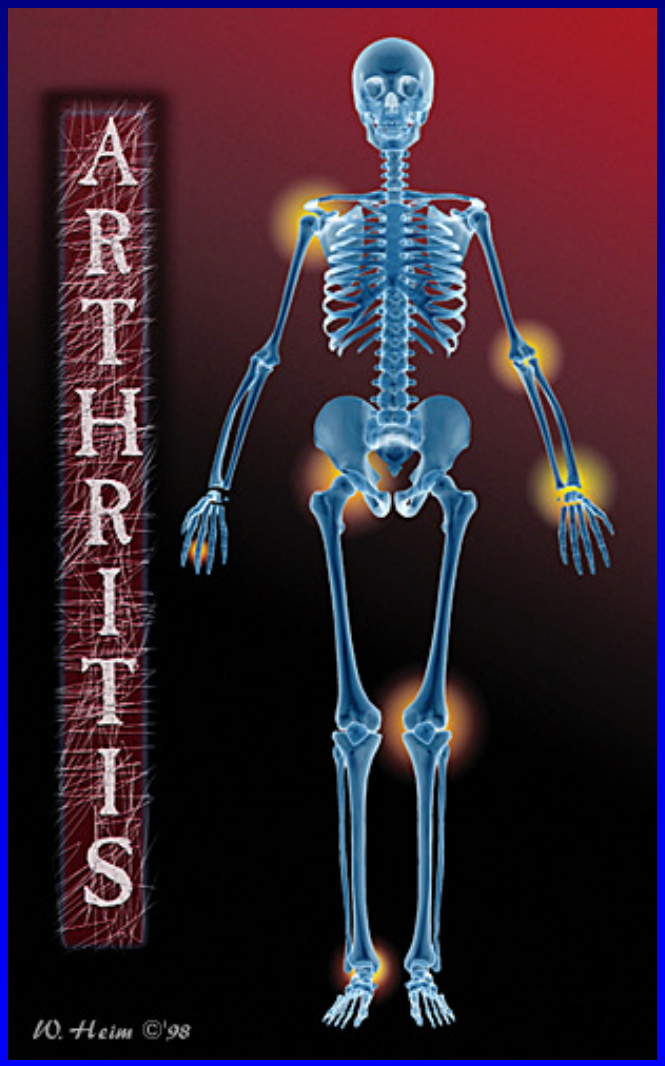
The Sympathetic Nervous System (SNS)



A not so "sympathetic" regulator of immune function in autoimmune disease:

RA as an example

Dianne Lorton ‡
Denise Bellinger ¤

Kent State University‡
Loma Linda University¤

3rd International Conference on Clin, & Cell. Immunol, Sept 23- Oct. 1, 2014

Neural-Immune Cross-Talk

Blood Borne Signals TNF, IL-1, IL-6

Sympathetic Nervous System

Pathogens

Bone Marrow

Thymus

Lymph Node

Lymphocytes Macrophages Dendritic cells Others

Spleen

GALT

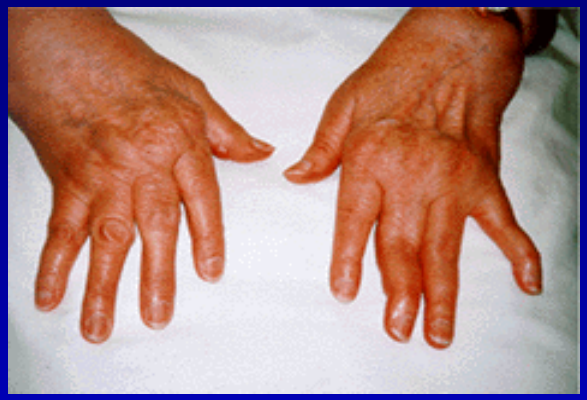
Blood

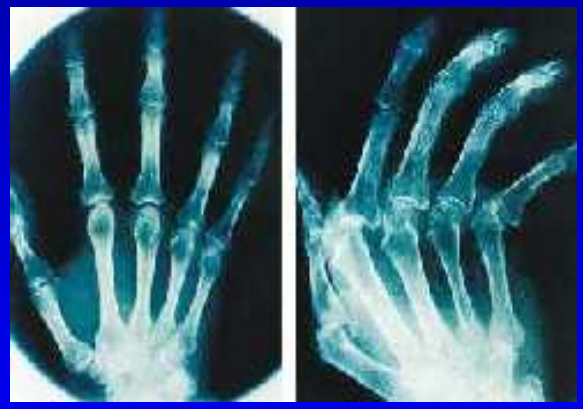
X

Disease

Rheumatoid Arthritis

- Autoimmune Disease
  - Chronic inflammatory response
  - Production of autoantibodies
  - Loss of Tolerance: Imbalance between autoreactive effector T cells (CD4+ Th1 & Th 17) and T reg cells
- Th cell balance regulated by the SNS
- SNS activity is chronically elevated in RA patients
- How this impacts Th cell balance is not known



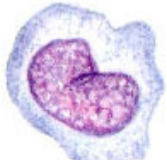


SNS Regulates Th Cell Differentiation via β^2-AR Activation of cAMP-PKA Pathway

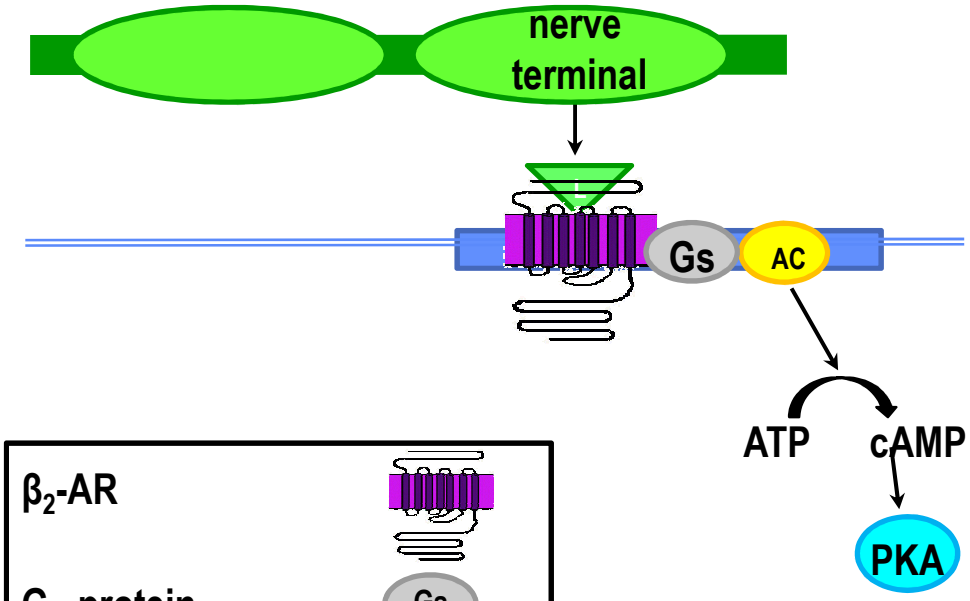
β_2

CD4+ β_2 (200-750 sites/cell)
CD4+ Th1 clones β_2 (250 sites/cell)
CD4+ Th2 clones (no detectable β_2)
CD4+ Treg cell [1]?
CD4+ Th17 cells?

APCs

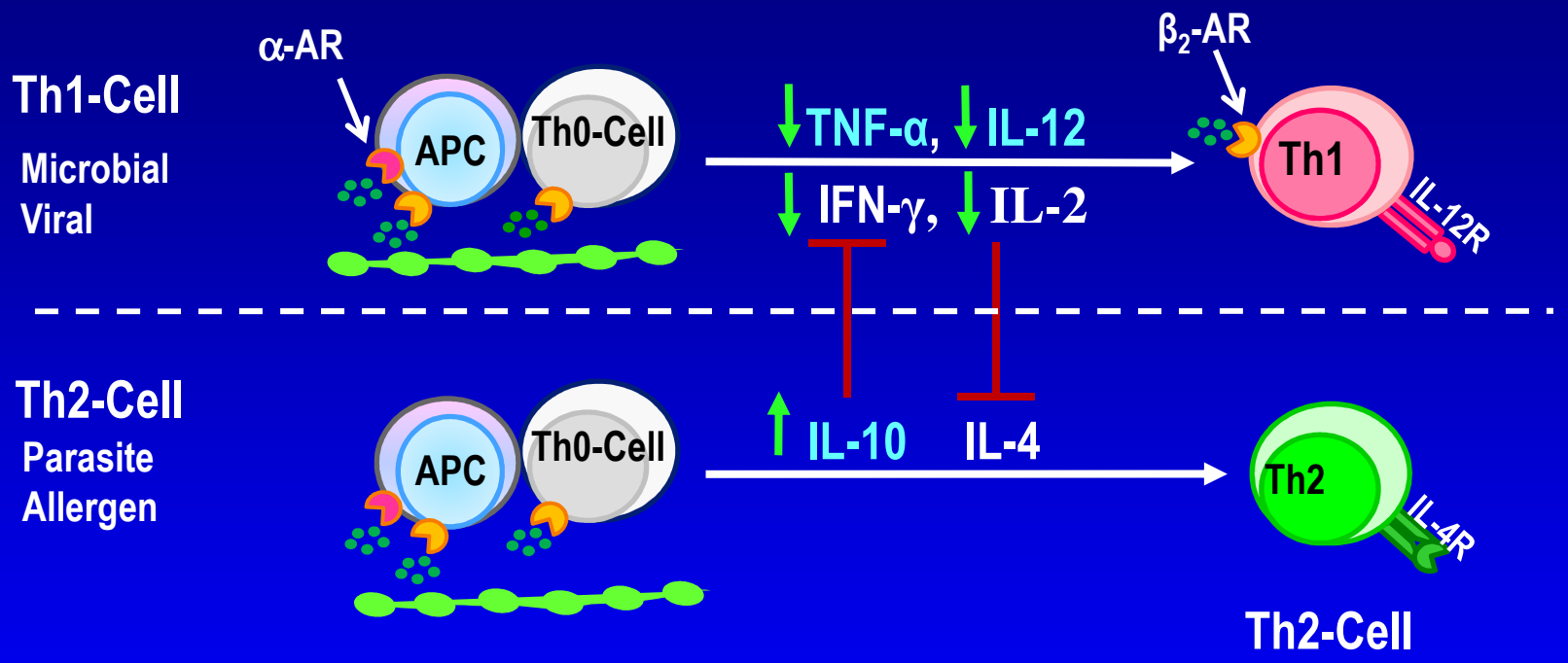


α, α_1, α_2, β, β_2



[1]Guereschi et al. Eur J Immunol. 2013 Apr;43(4):1001-12.

β_2-AR Shifts Th0 cell → Th2 Differentiation



Tregs?
Th17?

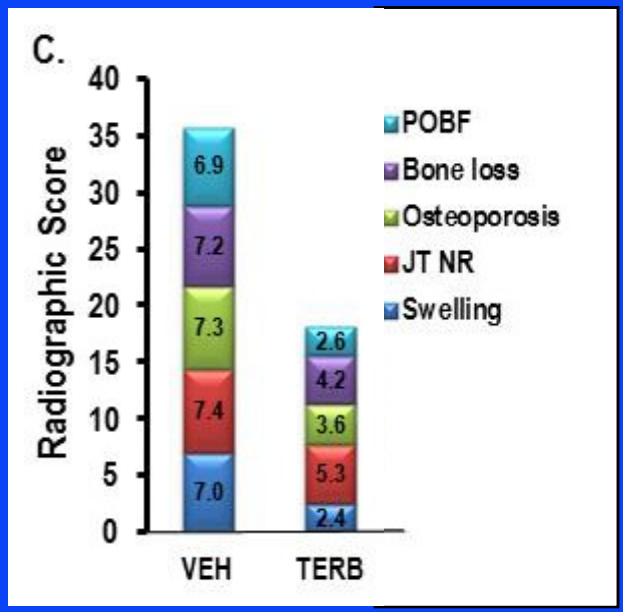
Guereschi et al., 2013

Hypothesis: Reduce disease severity is due in part to a β_2-AR driven shift in Th1 vs Th2 cell balance.

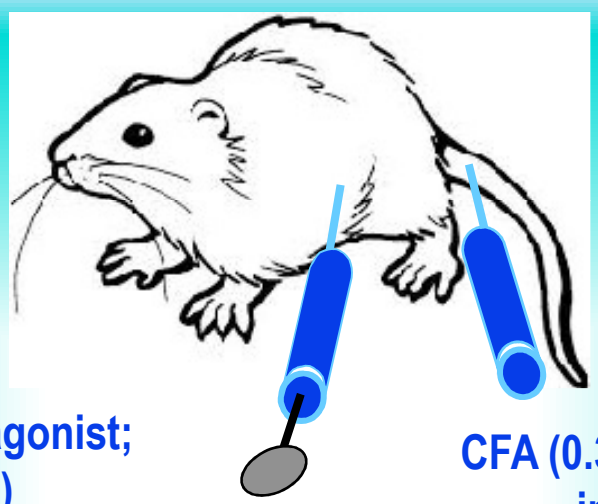
Challenge **Disease**

Ag Processing/ Clearance

(AA: Lorton et al., 1998; 2004)
(CIA: Malfait et al., 1999; Härle et al., 2005



Experimental Design



Adjuvant-Induced Arthritis (AA)

Day 12-28

Terbutaline (β_2-AR agonist; 1.5 mg/ml/day i.p.)
Saline Vehicle

Day 0

CFA (0.3 mg M. butyricum in 100 μl MO)

Day 28

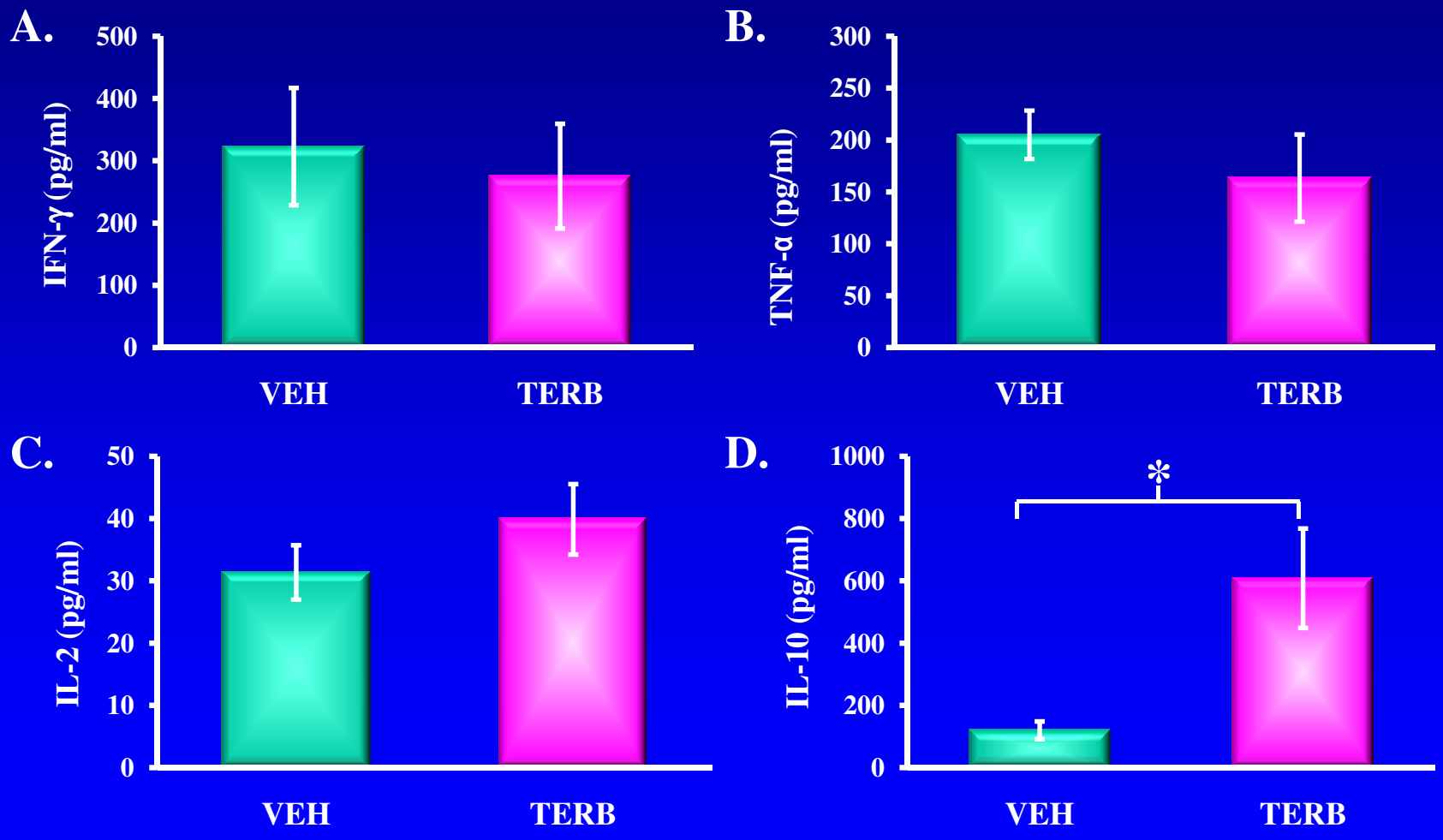
Spleen PBMCs

Draining Lymph Nodes (DLN)

Outcome Assessments

- Th1/2 cell cytokines (ELISAs)
- Foot Pad Swelling (data not shown)
- X-ray analysis (data not shown)

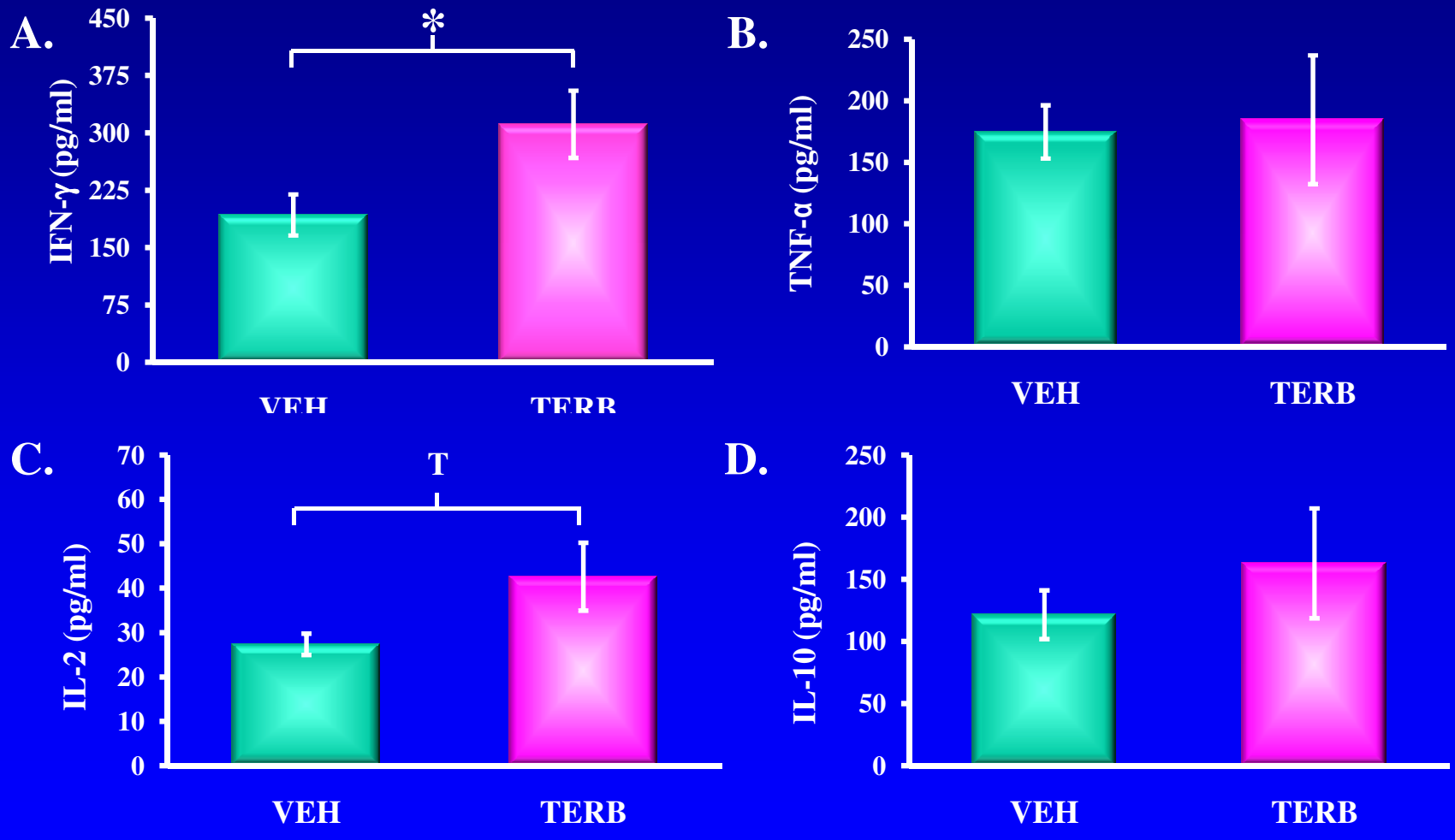
Spleen: Failure of a β_2-AR agonist to shift from a Th1 to Th2 cytokine profile



No Change in IL-4; (40-80 pg/ml)

Anova with Bonferoni post-hoc test, N = 8

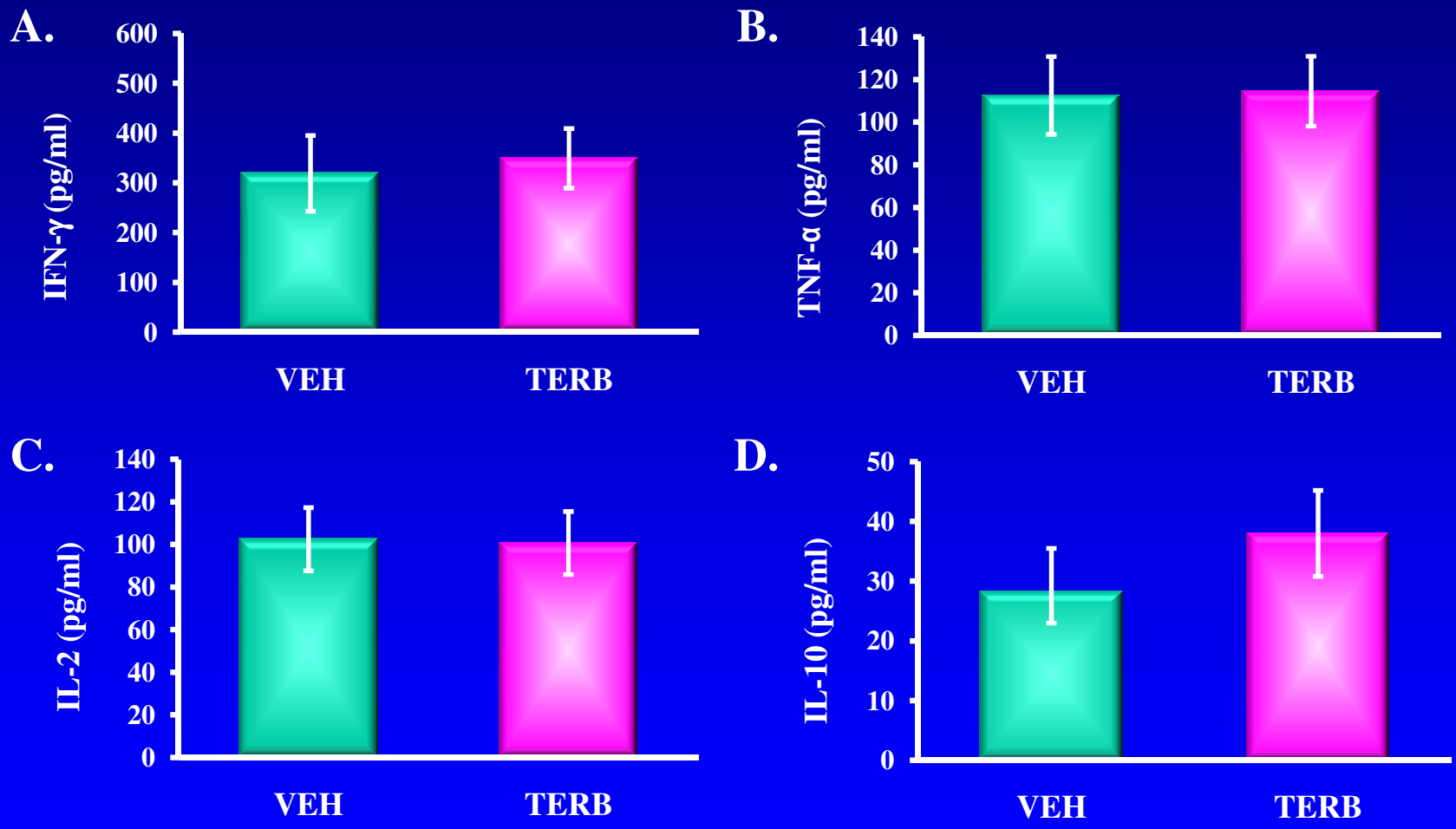
DLN: β_2-AR agonist promotes a Th1 cytokine profile



No Change in IL-4 (40 -80 pg/ml)

Anova with Bonferoni post-hoc test, N = 8; *P<0.05

PBMC: Failure of a β_2-AR agonist to shift Th1 cytokine profiles



Wahle et al., 2006. Failure of catecholamines to shift T-cell cytokine responses toward a Th2 profile in patients with rheumatoid arthritis. Arthritis Res. Ther., 8(5):R138.

Conclusions

- Different responses in each tissue examined: animal models critical for understanding RA
- Stimulating β_2-ARs after disease onset fails to inhibit Th1 cell driving cytokines
  - Spleen: β_2-AR agonists produced no change IFN-γ, IL-2, IL-4 or TNF-α, and increased IL-10 (source ?)
  - DLN stimulating β_2-ARs promotes IFN-γ & IL-2, no change in IL-4, IL-10, TNF-α
- β_2-AR stimulation under normal circumstances inhibits IFN-γ and IL-2 production via cAMP-PKA
- These findings indicate abnormal β_2-AR functions

Conclusions

- In spleen cells, the inability of terbutaline to reduce IFN-γ and IL-2 could be easily explained by the well-known down-regulation and desensitization of β_2-AR with repeated stimulation.
- Subsequent, cAMP assays and receptor binding experiments, confirmed this hypothesis (Lorton et al., Clin Dev Immunol., 2013)
- However, the terbutaline-induced increase in IFN-γ and IL-2 were intriguing. → not explained by canonical signaling of β_2-AR

Does Altered β2-AR Coupling to Second Messengers Occur in DLNs in AA: cAMP-PKA to ERK1/2?

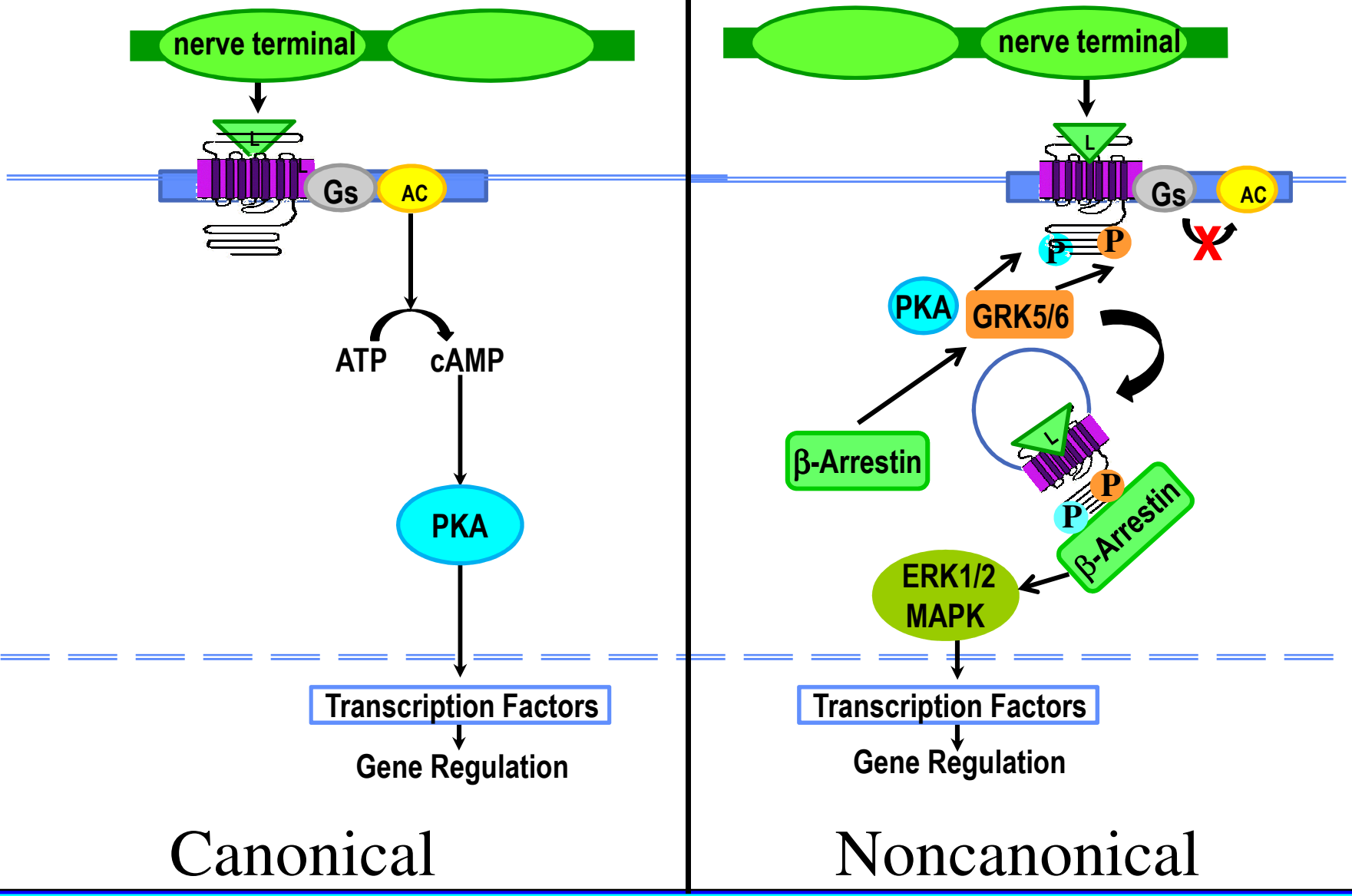
THE JOURNAL OF BIOLOGICAL CHEMISTRY VOL. 281, NO. 2, pp. 1261–1273, January 13, 2006

β-Arrestin-dependent, G Protein-independent ERK1/2 Activation by the β2 Adrenergic Receptor*

Received for publication, June 16, 2005, and in revised form, November 2, 2005 Published, JBC Papers in Press, November 9, 2005, DOI 10.1074/jbc.M506576200

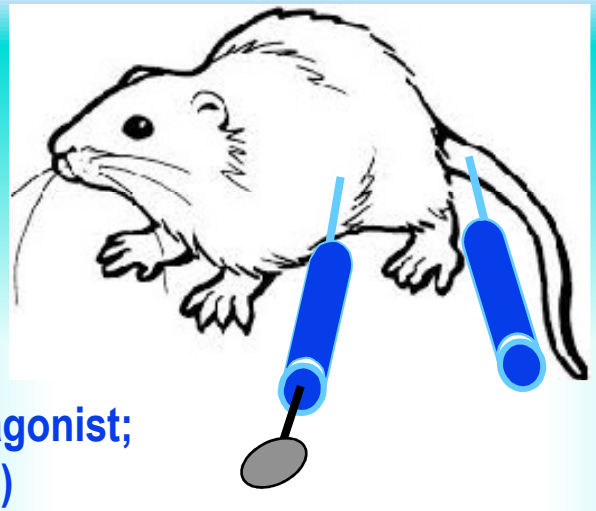
Sudha K. Shenoy‡1,Matthew T. Drake‡2, Christopher D. Nelson‡, Daniel A. Houtz‡, Kunhong Xiao‡, Srinivasan Madabushi§, Eric Reiter‡¶, Richard T. Premont‡, Olivier Lichtarge§, and Robert J. Lefkowitz‡3

Altered Receptor Signaling in the DLN?



Hypothesis: Terbutaline induces a shift in β_2-ARs signaling from cAMP-PKA to ERK 1/2 in the DLN

Adjuvant-Induced Arthritis (AA)



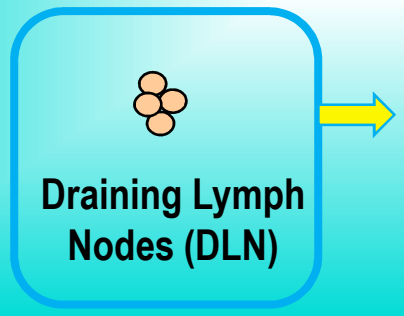
Day 1

CFA (0.3 mg M. butyricum in 100 µl MO)
Mineral Oil (MO)
M. Butyricium (in saline; SMB)
Saline

Day 12-28

Terbutaline (β_2-AR agonist; 1.5 mg/ml/day i.p.)
Saline Vehicle

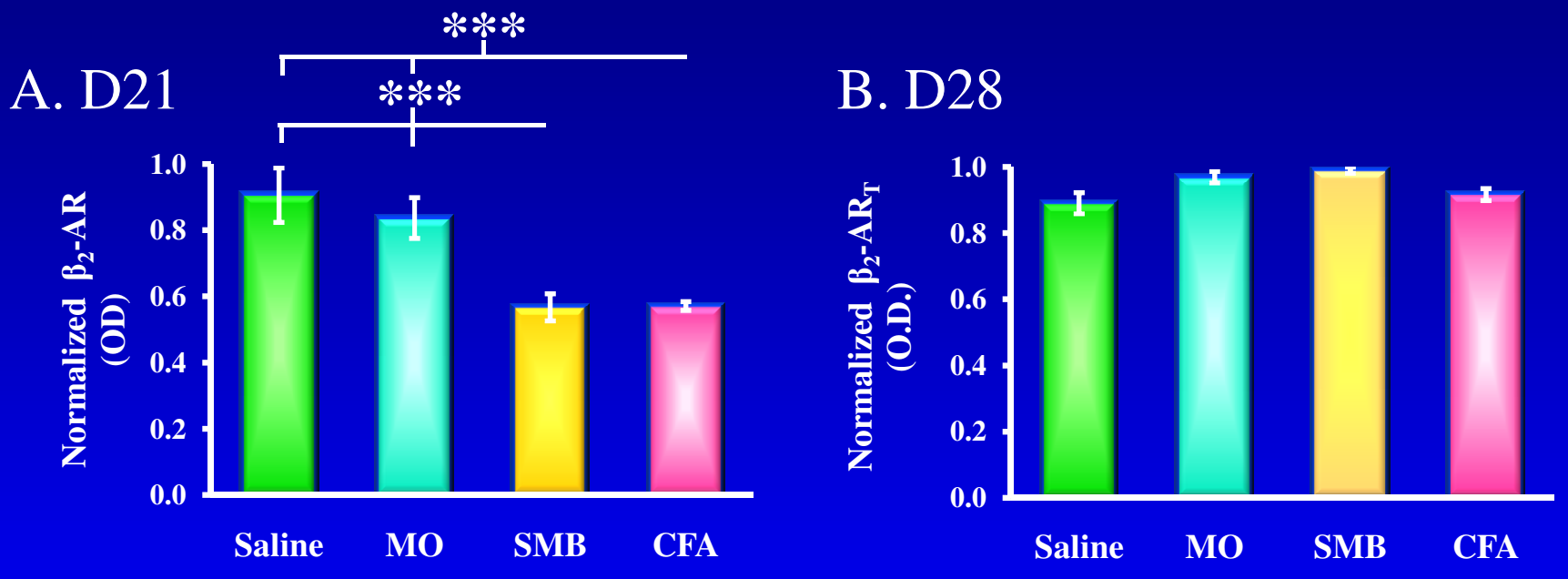
Day 21 or 28

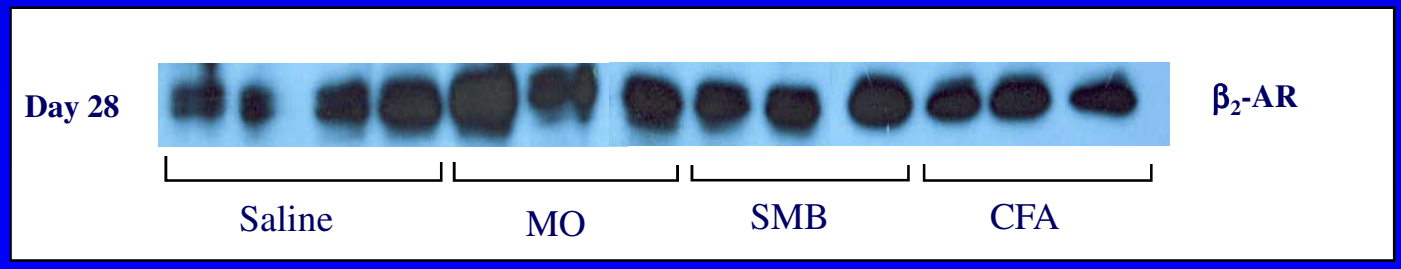


Outcome Assessments

DLN: β_2-AR Western Blots (antibodies to detect β_2-ARs, and β_2-ARs phosphorylated by PKA and or GRK)

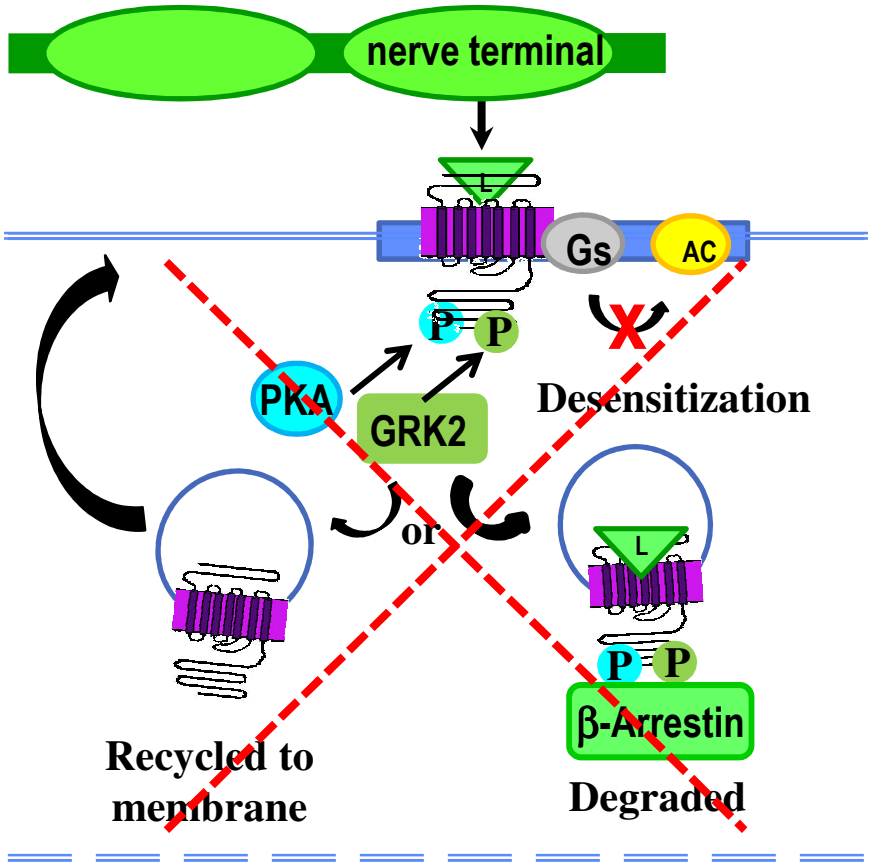
Unchanged DLN β_2-AR Density Late Disease





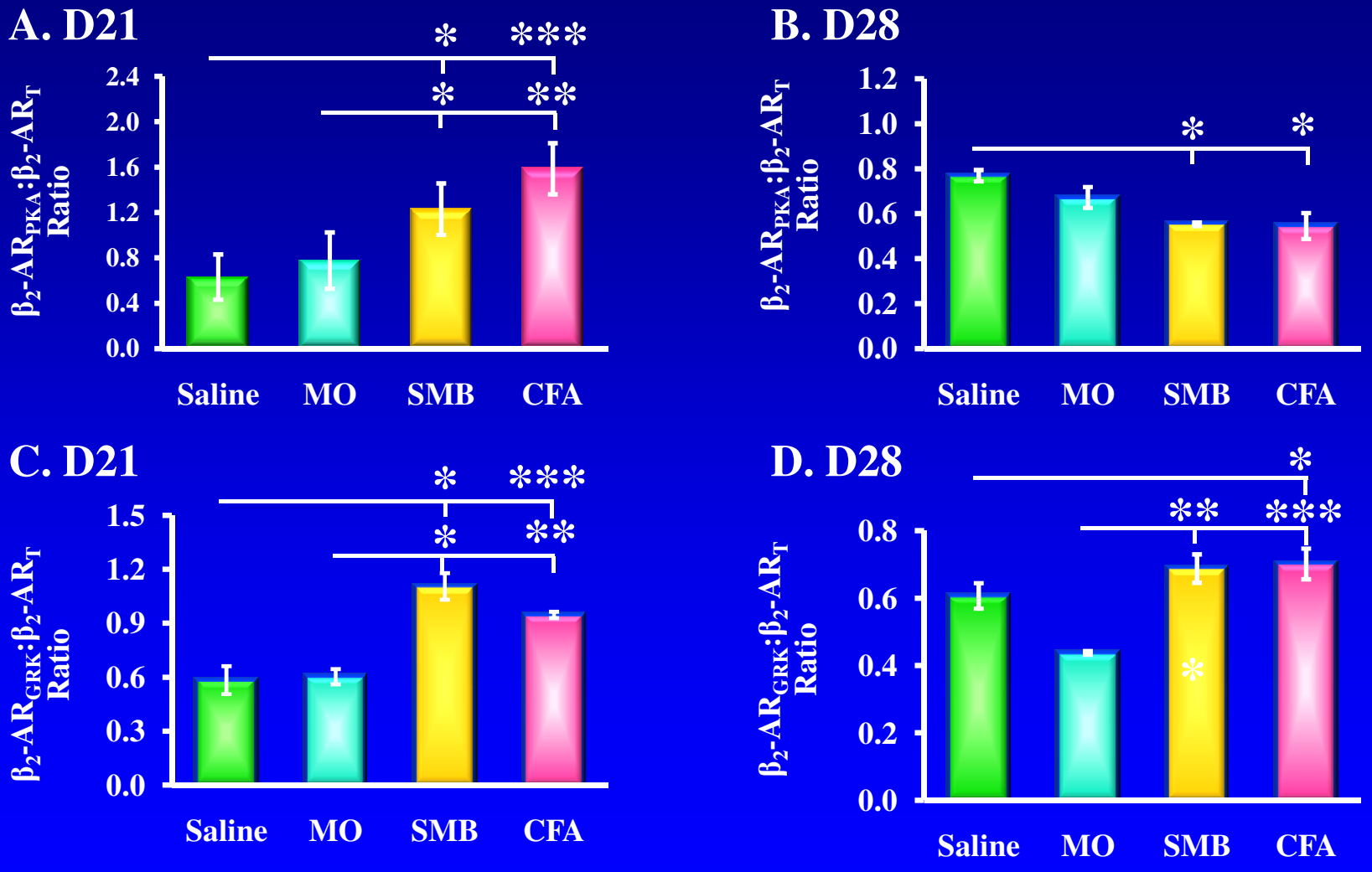
ANOVA; Bonferoni Post-Hoc Test N=4; *P < 0.05; **P < 0.01, ***P<0.001

Altered Receptor Signaling in the DLN?



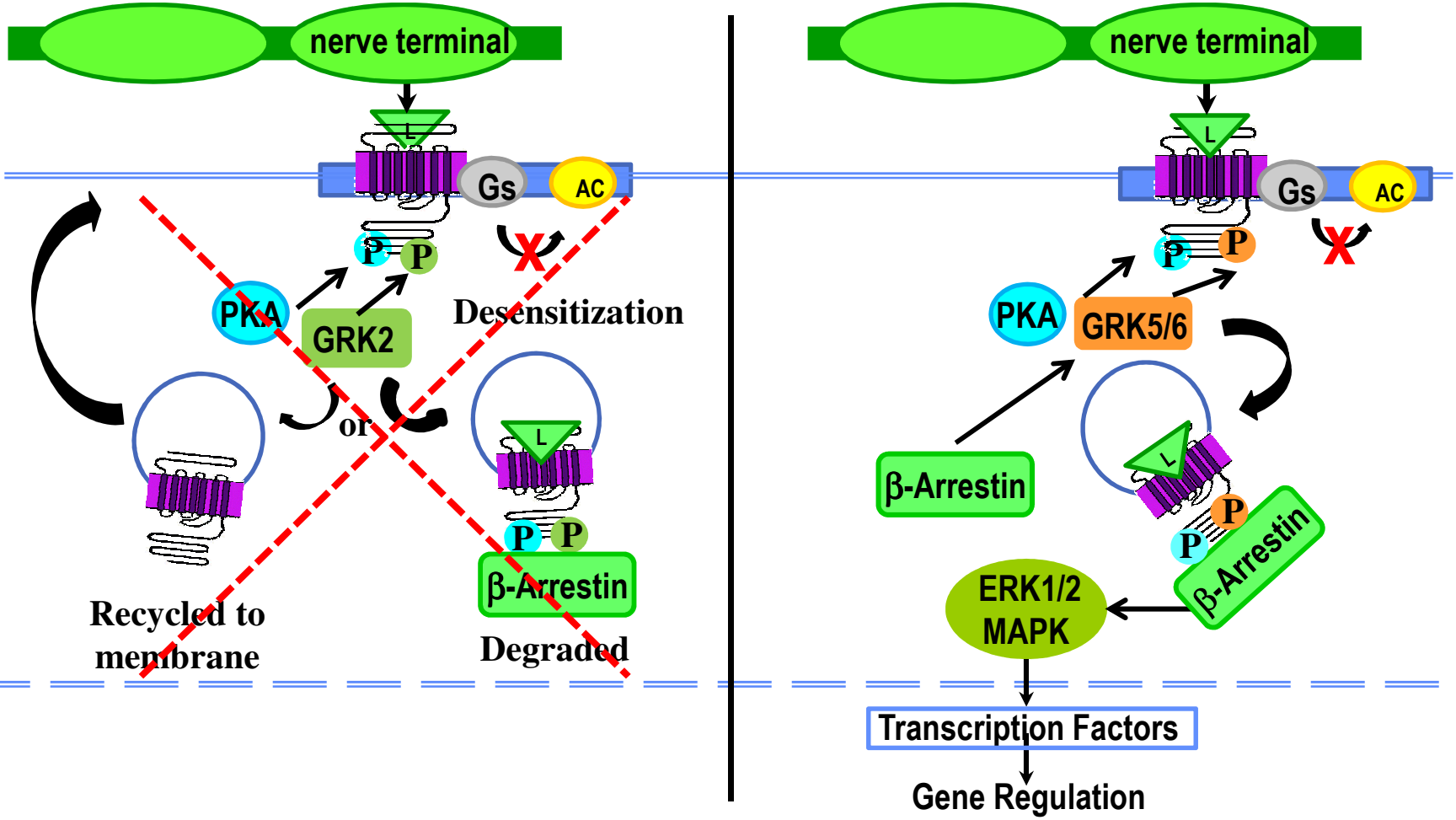
Conclusions: These findings along with increased IFN-γ indicate that β_2-ARs in DLN are <u>NOT</u> down-regulated or desensitized.

β_2-AR phosphorylated by PKA and GRK in DLN



ANOVA; Bonferoni Post-Hoc Test N=4; *P < 0.05; **P < 0.01, ***P<0.001

Altered Receptor Signaling in the DLN?



Conclusions: These findings coupled with increased IFN-γ, provide support β_2-AR signaling via ERK1/2.

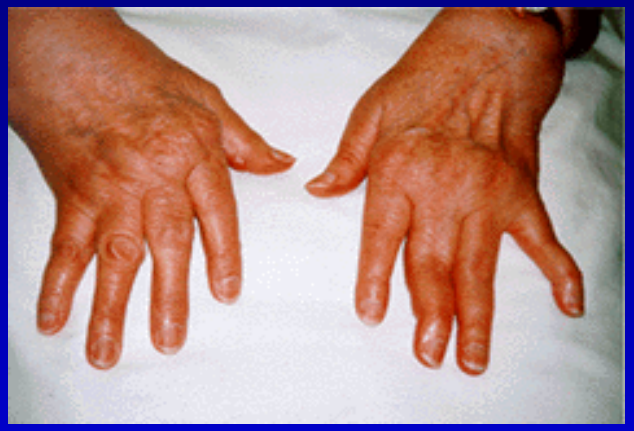
Summary

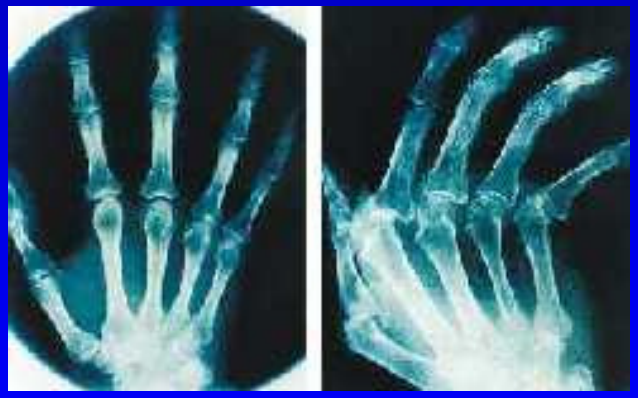
- Findings support a shift in β_2-AR receptor signaling from cAMP-PKA to ERK1/2 in DLN
  - β_2-AR agonist elevated IFN-γ and IL-2
  - No change in β_2-AR density,
  - Receptor phosphorylation by PKA increased PKA (day 21) and GRK phosphorylation (day 21 and 28)

Future Studies

- Are GRK5/6 and ERK 1/2 elevated in DLN cells?
- Can production of IFN-γ be blocked by inhibitors of ERK1/2 pathway?
- Why the different profiles in the spleen and DLN?
  - Inflammatory cytokine levels
  - CFA distribution/concentration
- Does the SNS regulate balance between Th17 and Treg cells?

Acknowledgements





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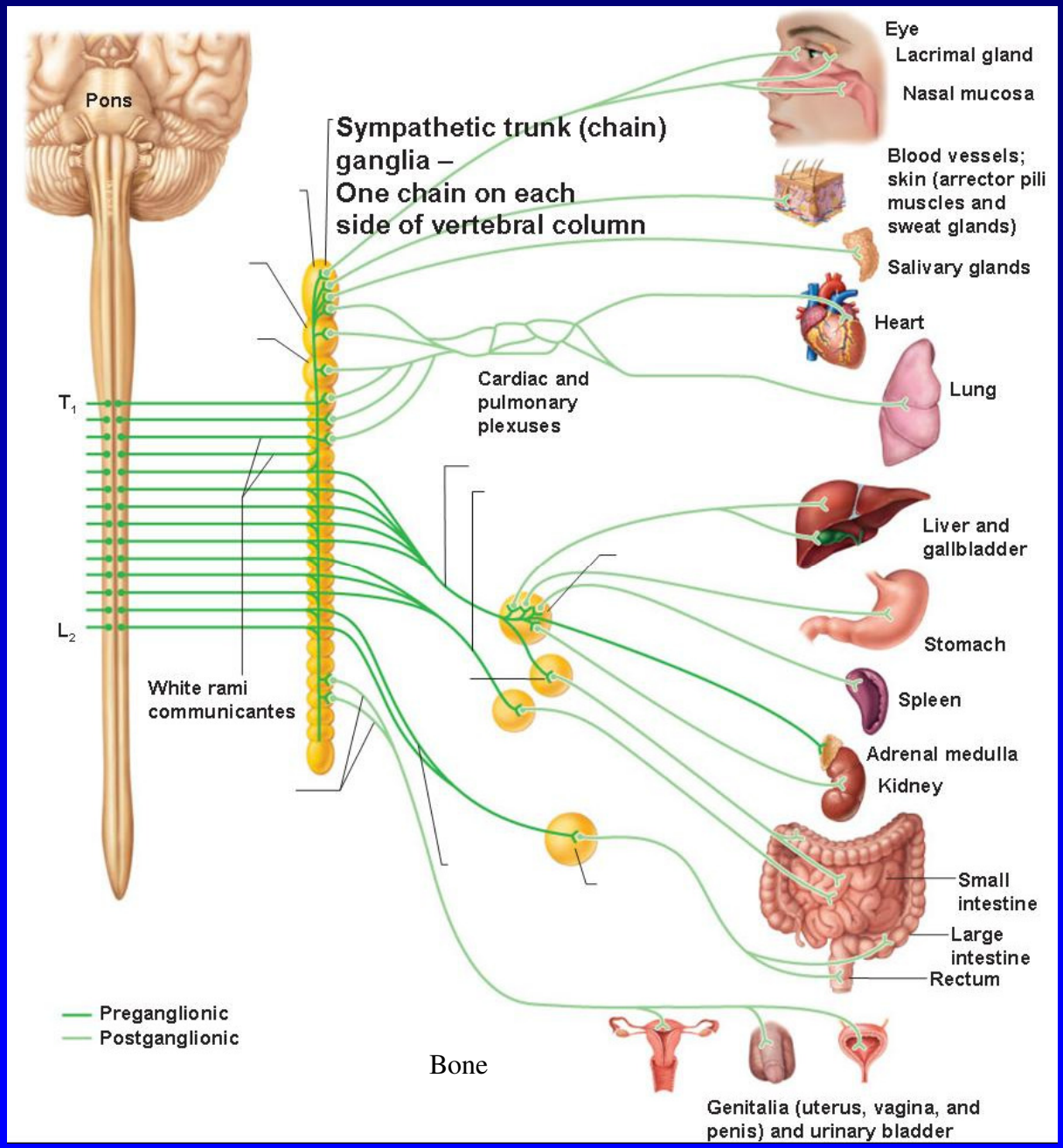
SNS Function: Respond to stress & maintain normal body functions (homeostasis)

The SNS integrates the functions of many systems required to mount an immune response



SNS Inhibition of Th1 Cytokines (IFN-γ and IL-2) to Push Th2 Cell Differentiation Occurs via β_2-AR of cAMP-PKA Pathway

T β_2

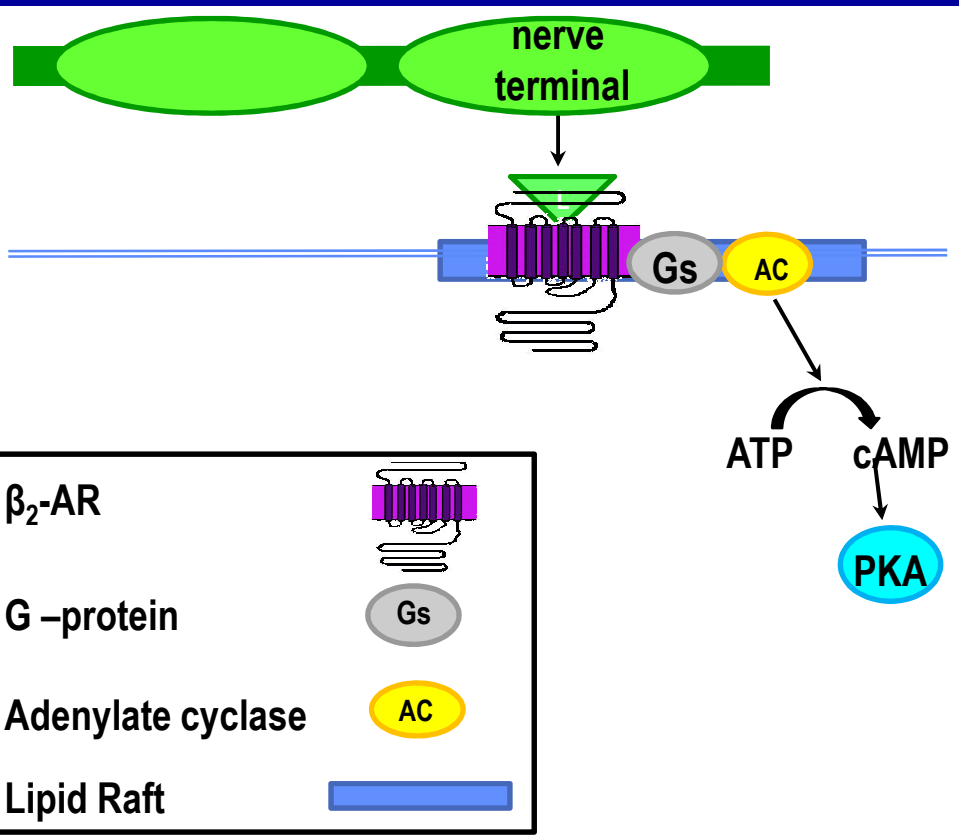
CD4+ β_2 (200-750 sites/cell)
CD8+ β_2 (500-2500 sites/cell)
CD4+ Th1 clones β_2 (250 sites/cell)
CD4+ Th2 clones (no detectable β_2)
CD4+ Treg cell [1]
CD4+ Th17 cells?

B

$\alpha, \alpha_1, \alpha_2, \beta, \beta_2$

macrophage

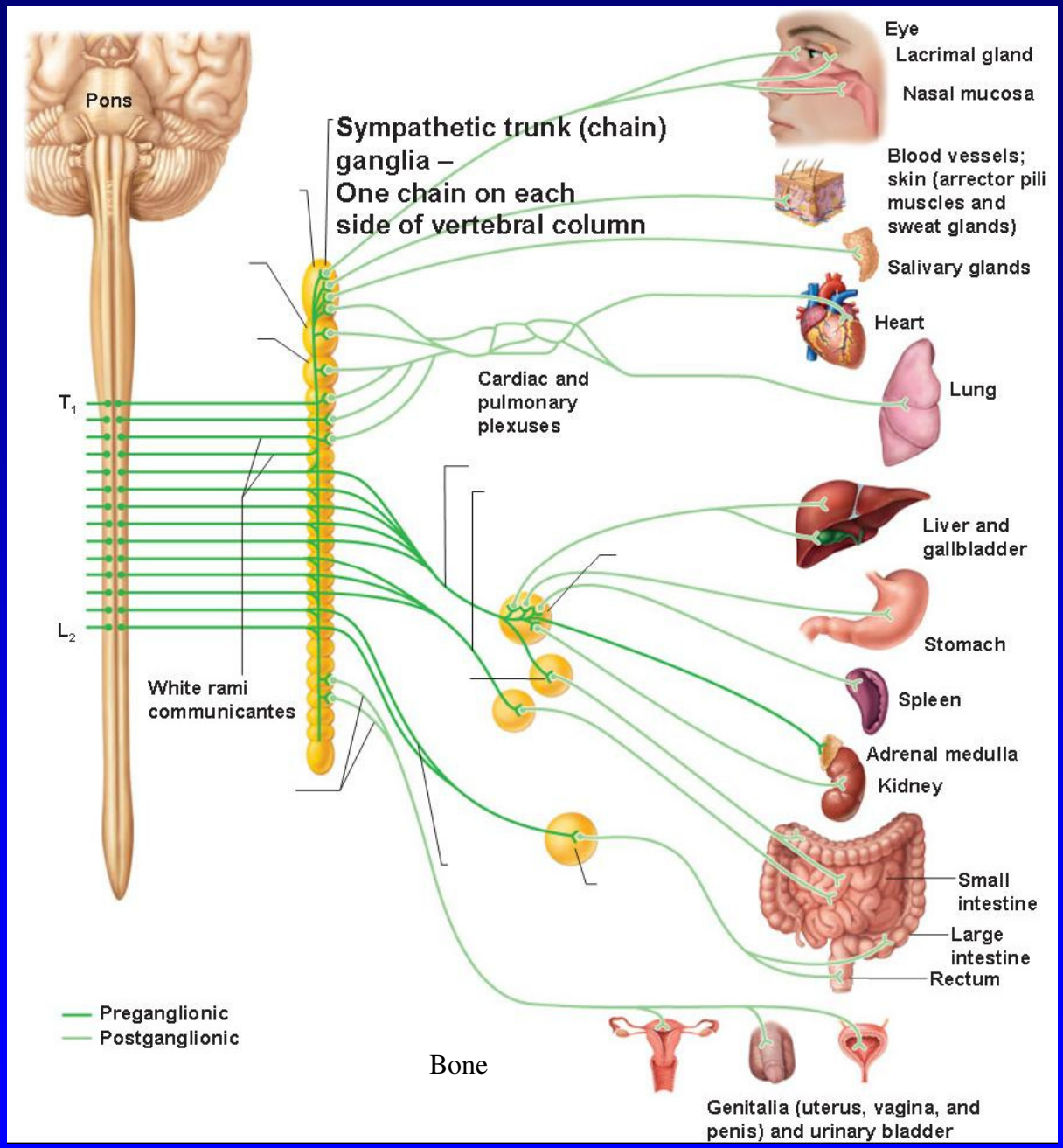
$\alpha, \alpha_1, \alpha_2, \beta, \beta_2$



[1]Guereschi et al. Eur J Immunol. 2013 Apr;43(4):1001-12.

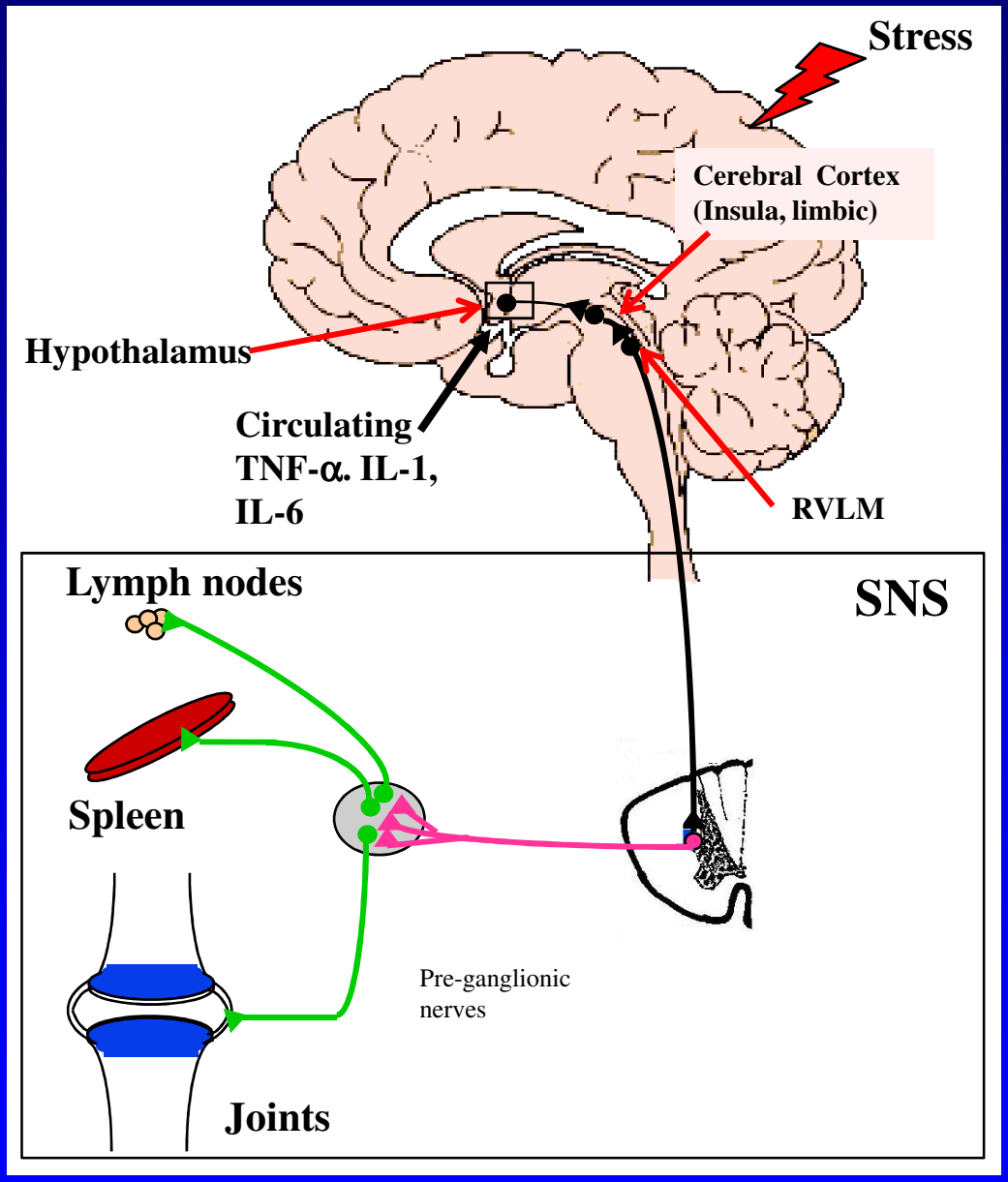
The SNS integrates the functions of many systems required to mount an immune response

Function:
Respond to stress
&
maintain normal body functions (homeostasis)
(allostasis- a new "adaptive" normal)

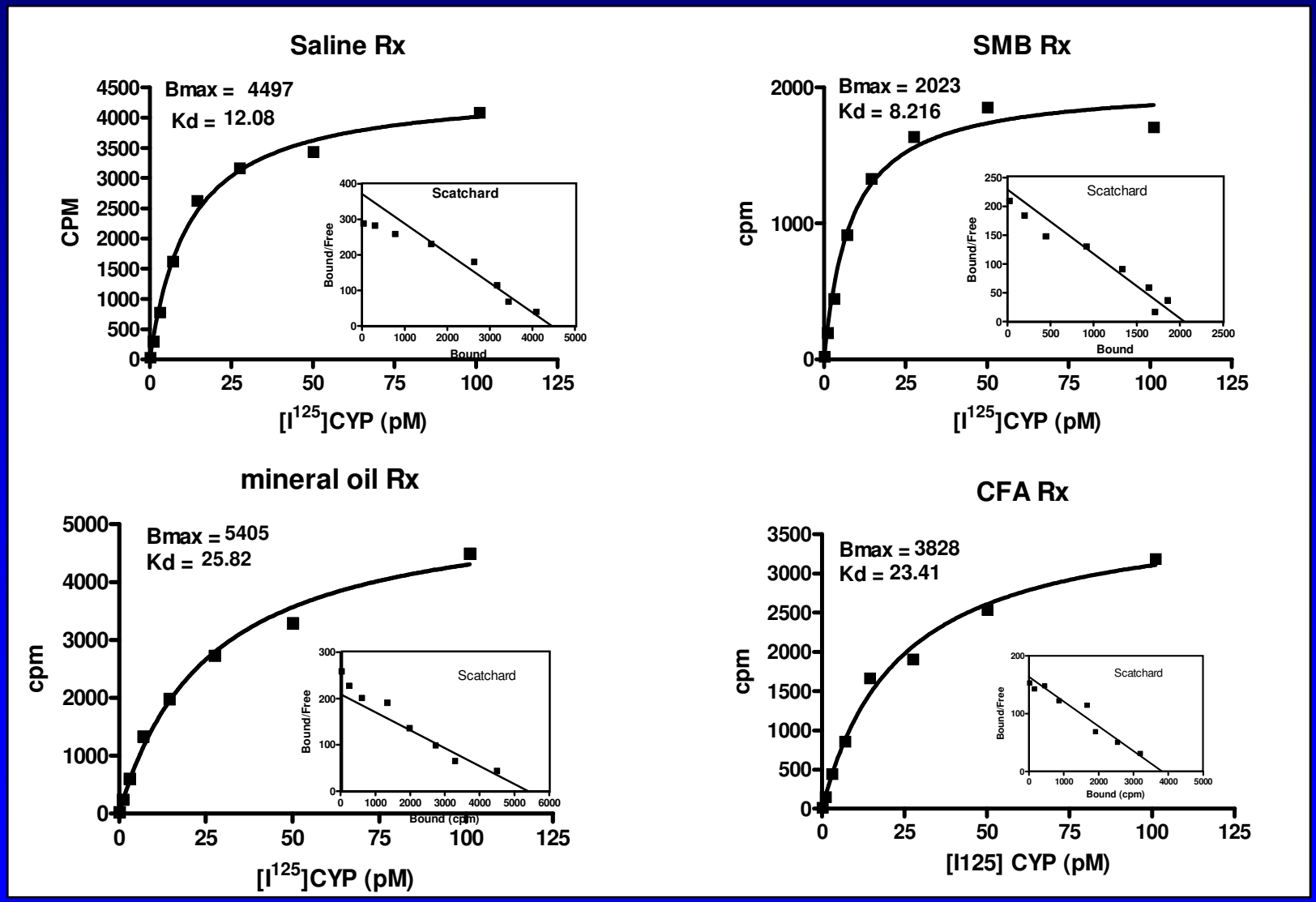


Reciprocal Immune System to SNS Communication in RA

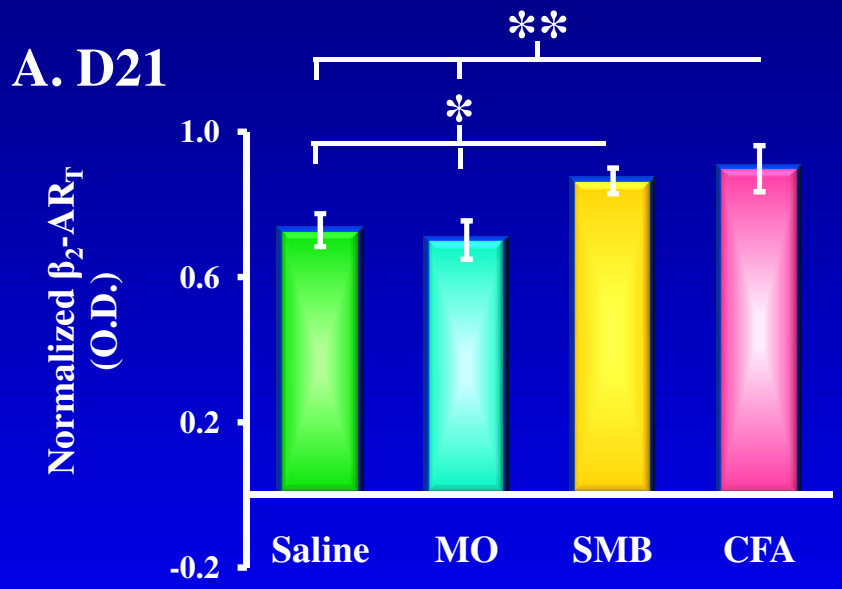
- Mechanism for emotional distress to impact health & disease
- ~ 80% of patients associate disease onset with a severe emotional life stressor (Trigger?)
- Stroke Victims: no RA in paralyzed limbs (↓ vs ↑ SNS nerve activity)

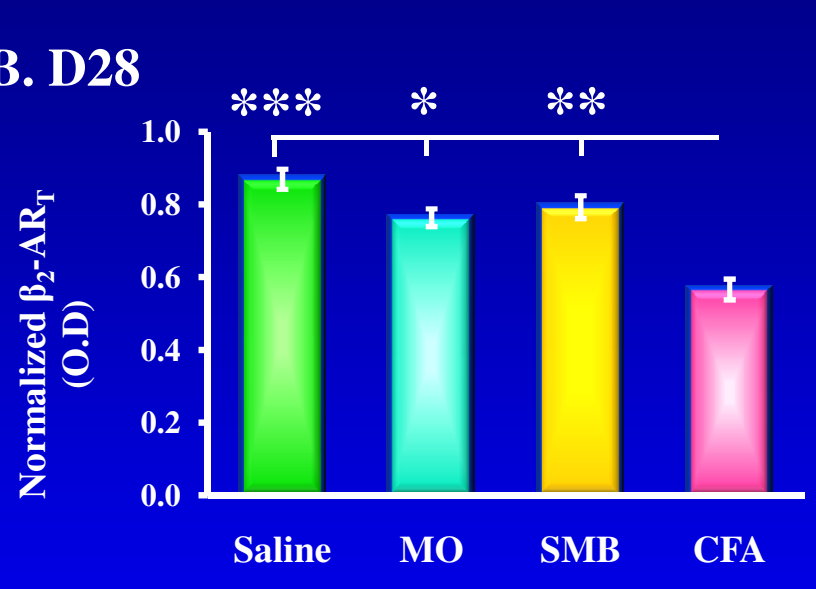


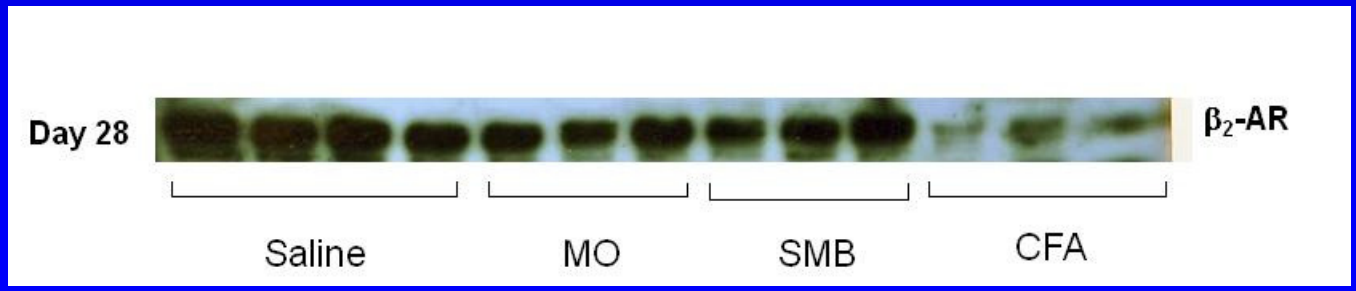
Splenocyte β_2-AR Receptor Binding in Arthritic Rats: Saturation Curves



SNS-IS Cross-Talk Pathology in RA:
Reduced Spleen β_2-AR Density Late Disease





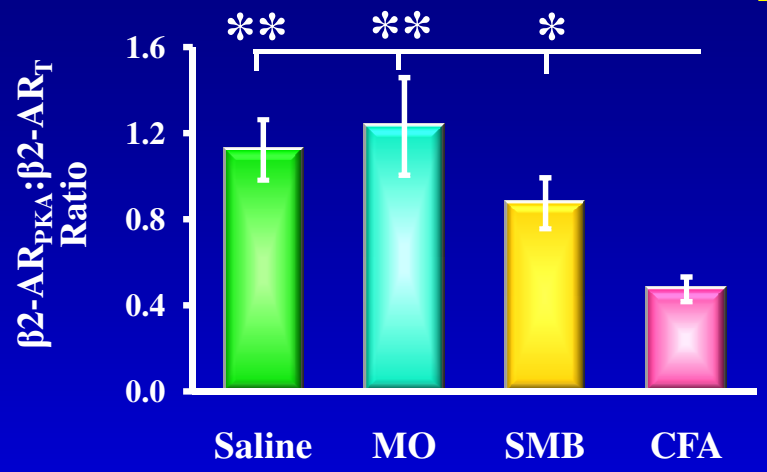


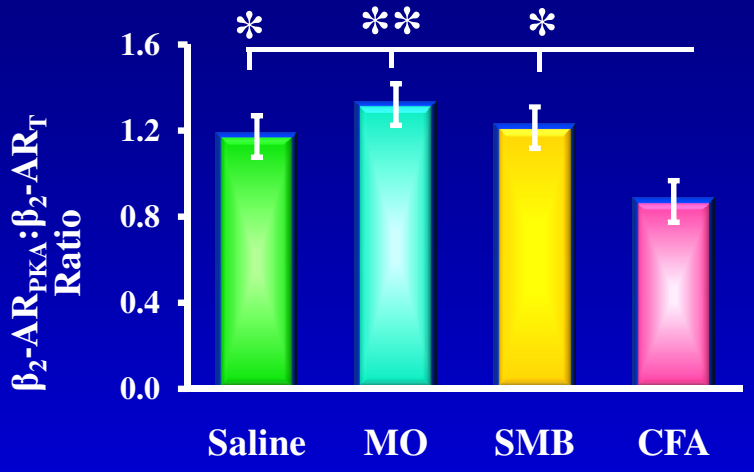
ANOVA; Bonferoni Post-Hoc Test

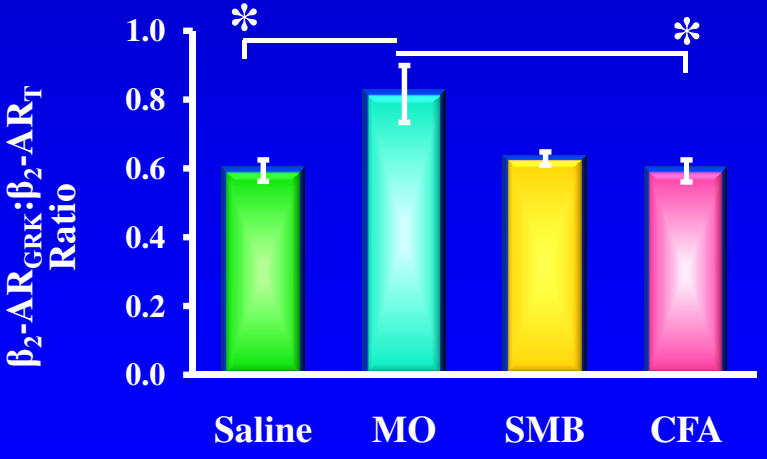
N=4; *P < 0.05; **P < 0.01, ***P<0.001

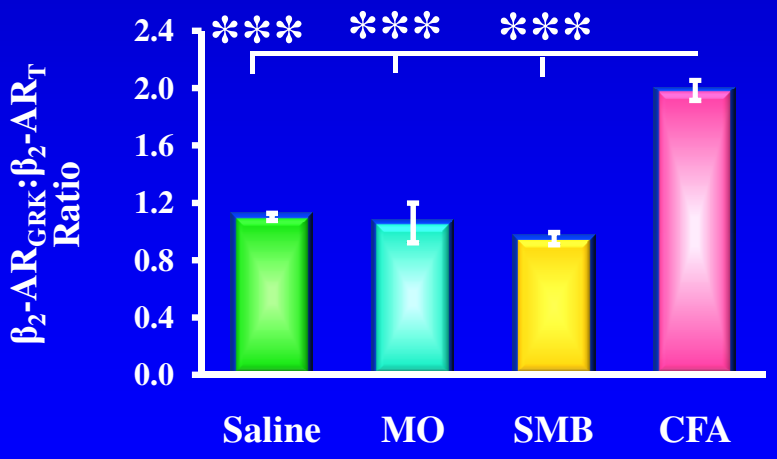
Lorton et al., (2013) Clin Dev Immunol.;2013:764395

β_2-AR Phosphorylation Patterns in the Spleen









ANOVA; Bonferoni Post-Hoc Test

N=4; *P < 0.05; **P < 0.01, ***P<0.001

Hypothesis: Chronic high SNS activity in RA induces β_2-AR down regulation and desensitization

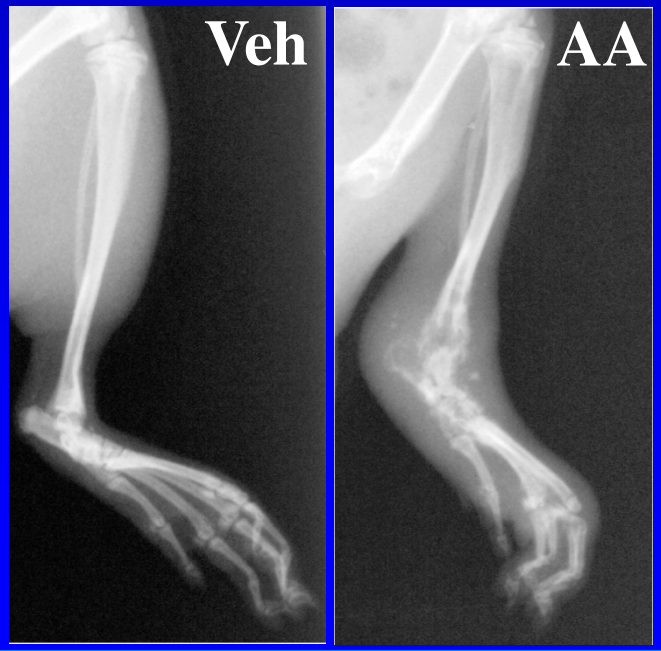
Day 1

[a]0.3 mg Mycobacterium butyricum in 0.1 ml sterile mineral oil

[a]CFA/ICA (vehicle)

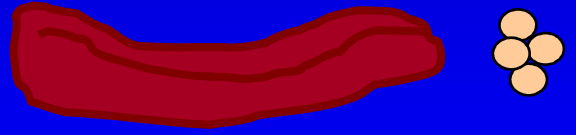
Autoantigen: HSP 65

Day 28



Day 21 or 28

Harvest Spleen & DLN cells

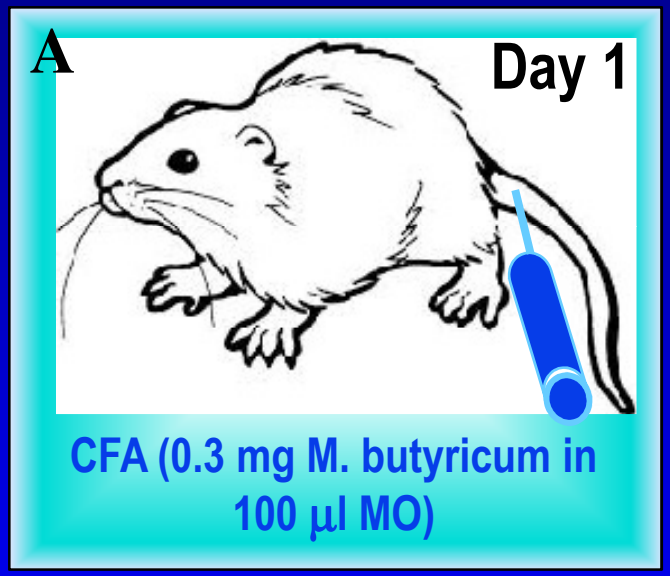


cAMP assay

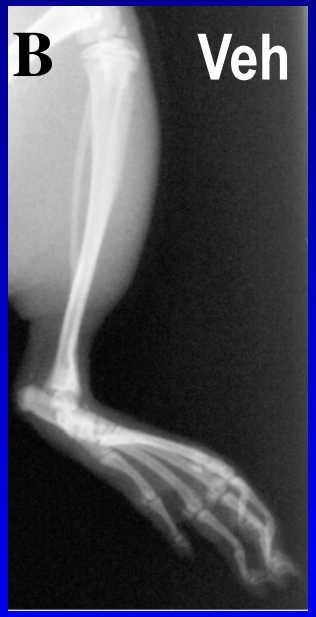
β_2-AR Receptor Binding Assays

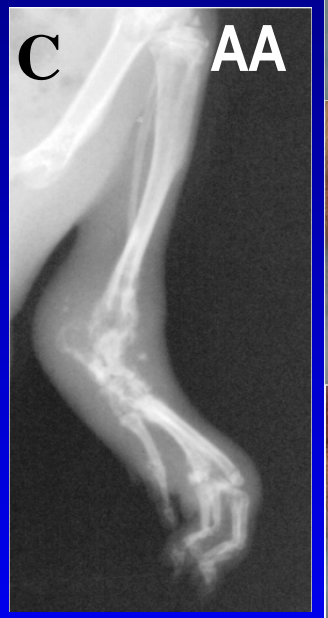
β_2-AR Western Blots
using antibodies to detect phosphorylated receptor

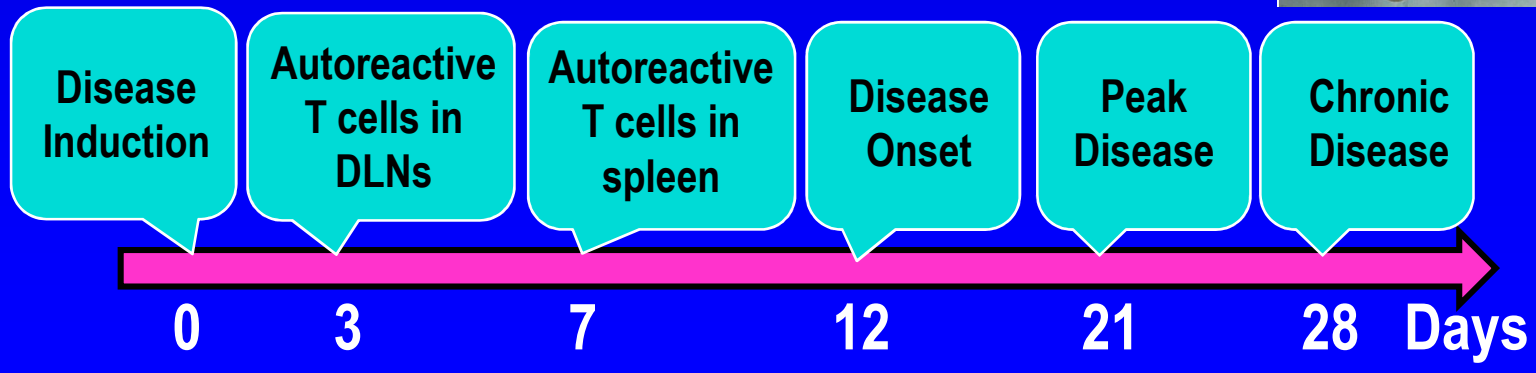
Adjuvant-Induced Arthritis Rat Model



Day 28

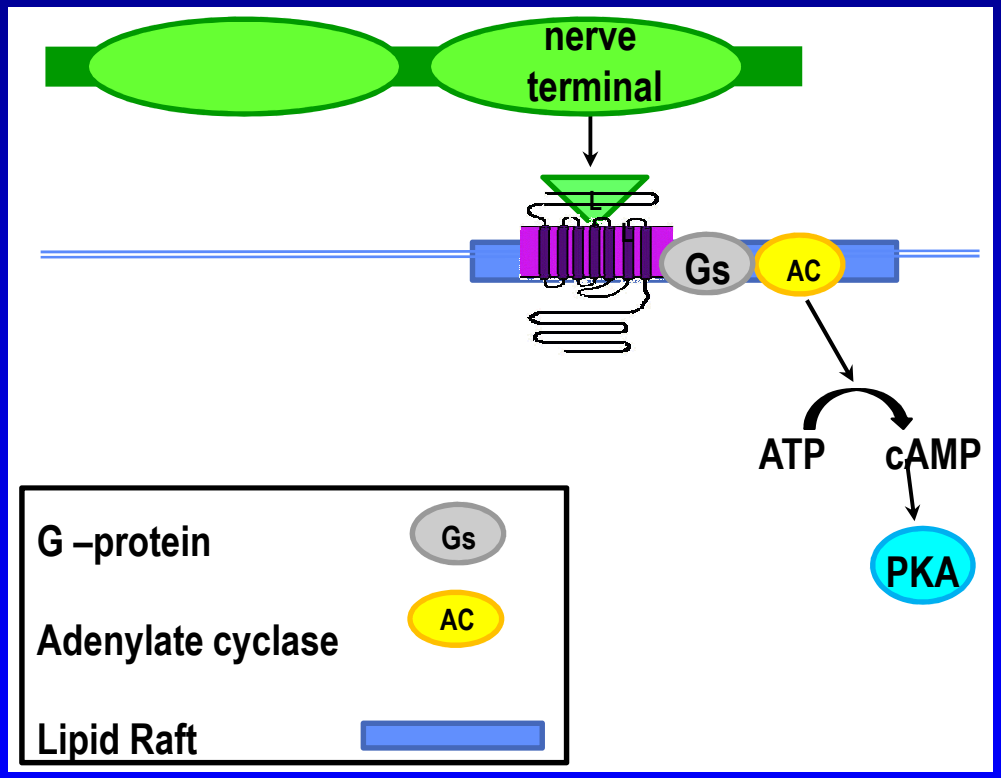


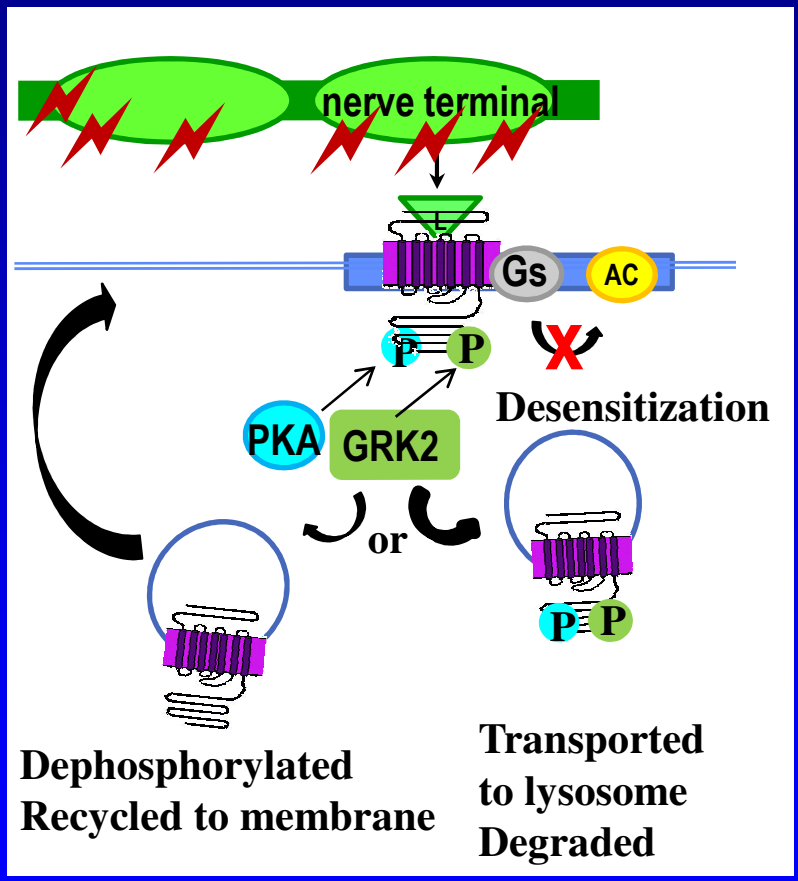




SNS-IS Cross Talk in the Spleen?

$ß_2$-AR & Signaling via the Canonical Pathway: cAMP





Hypothesis: Chronic high SNS activity in RA induces $β_2$-AR down regulation and desensitization in splenocytes

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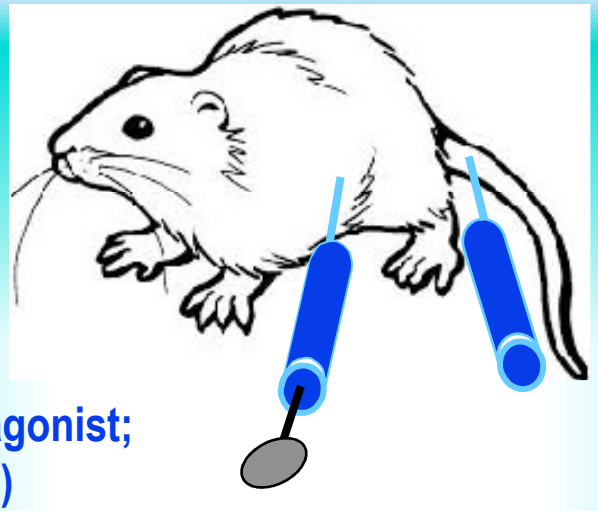


Day 1

CFA (0.3 mg M. butyricum in 100 μl MO)
Mineral Oil (MO)
M. Butyricum (in saline; SMB)
Saline

Day 12-28

Terbutaline (β_2-AR agonist; 1.5 mg/ml/day i.p.)
Saline Vehicle

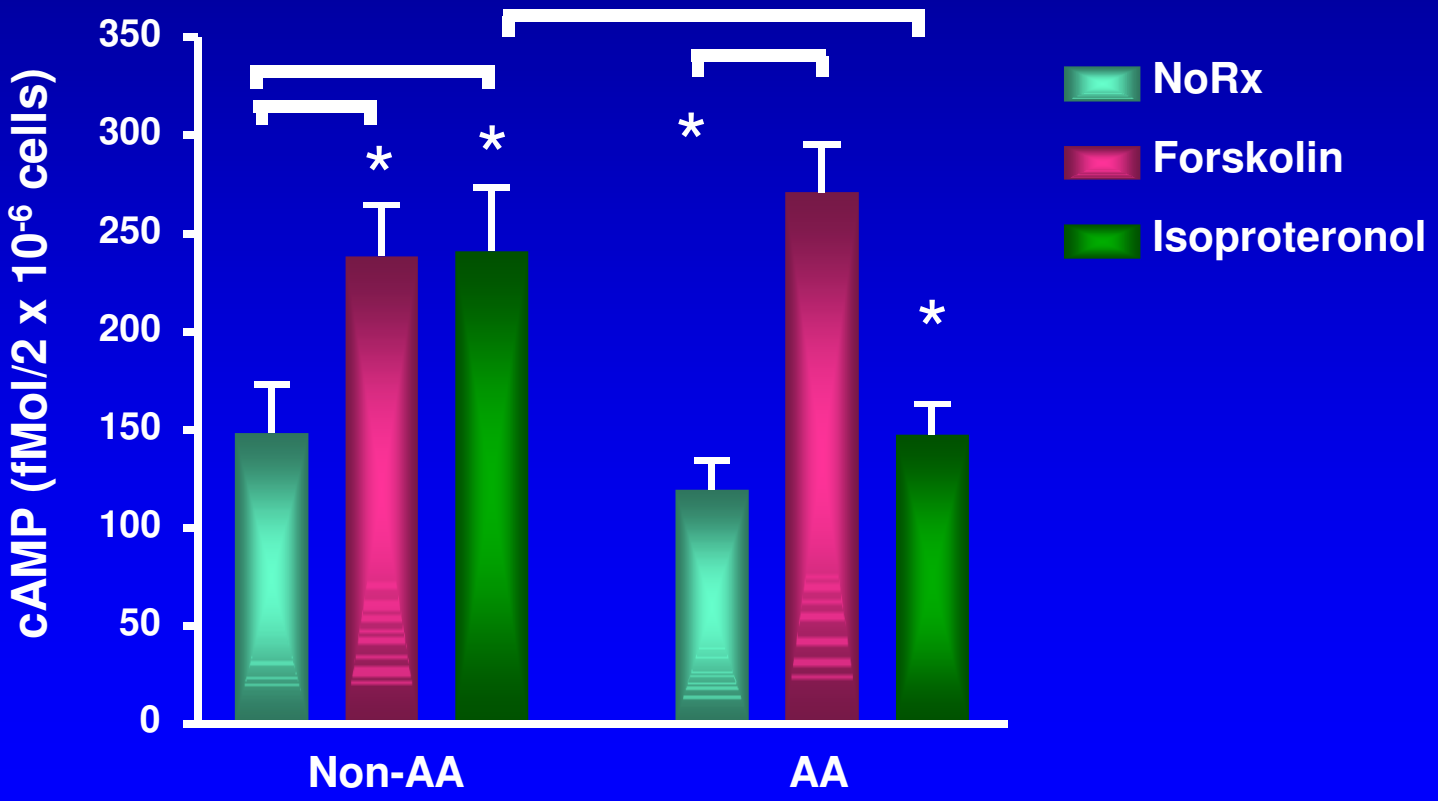
Day 21 or 28

Outcome Assessments

Spleen: cAMP assay (spleen)
β_2-AR Binding Assays
DLN: β_2-AR Western Blots
(antibodies to detect β_2-ARs)

SNS-IS Cross-Talk Pathology in RA:

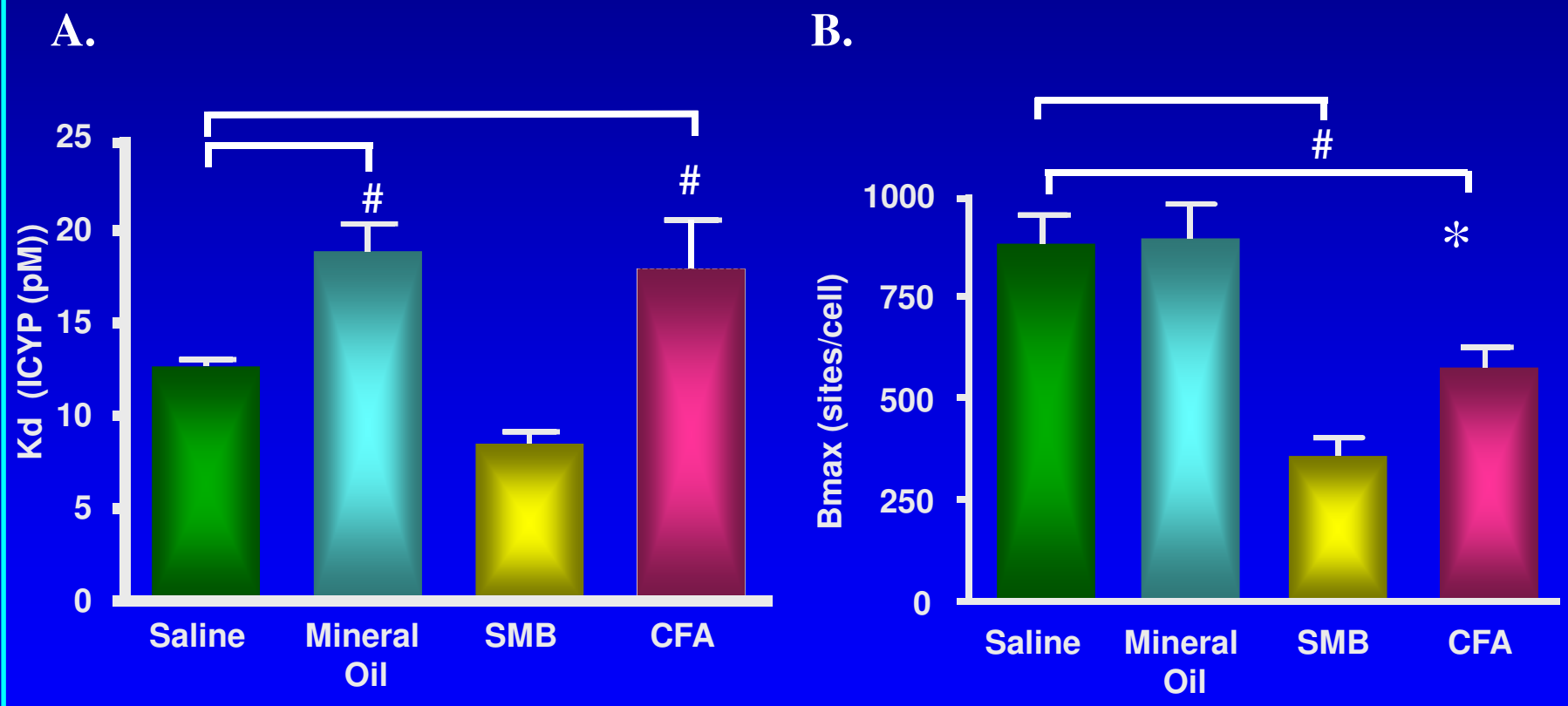
β_2-AR Agonist Fails to Induce cAMP in Splenocytes



Anova with Bonferoni post-hoc test,
Day 28; N = 8; *P<0.05

Lorton et al., (2013) Clin Dev Immunol.;2013:764395

SNS-IS Cross-Talk Pathology in RA:
Splenocyte β_2-AR have Reduced Agonist Affinity and Density

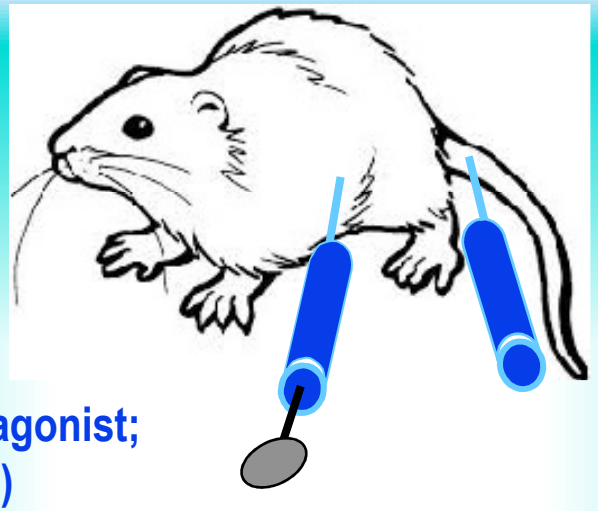


ANOVA; Bonferoni Post-Hoc Test

N=6; * P < 0.05; #P < 0.01

Lorton et al., (2013) Clin Dev Immunol.;2013:764395

Hypothesis: Chronic high SNS activity in RA induces β_2-AR down regulation and desensitization in the spleen



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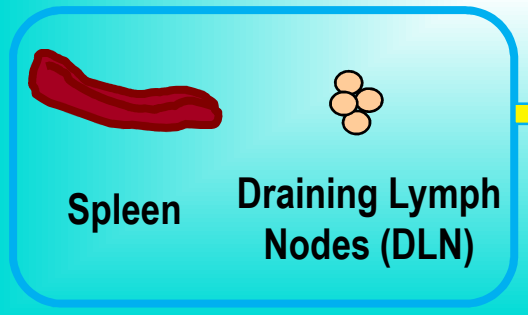

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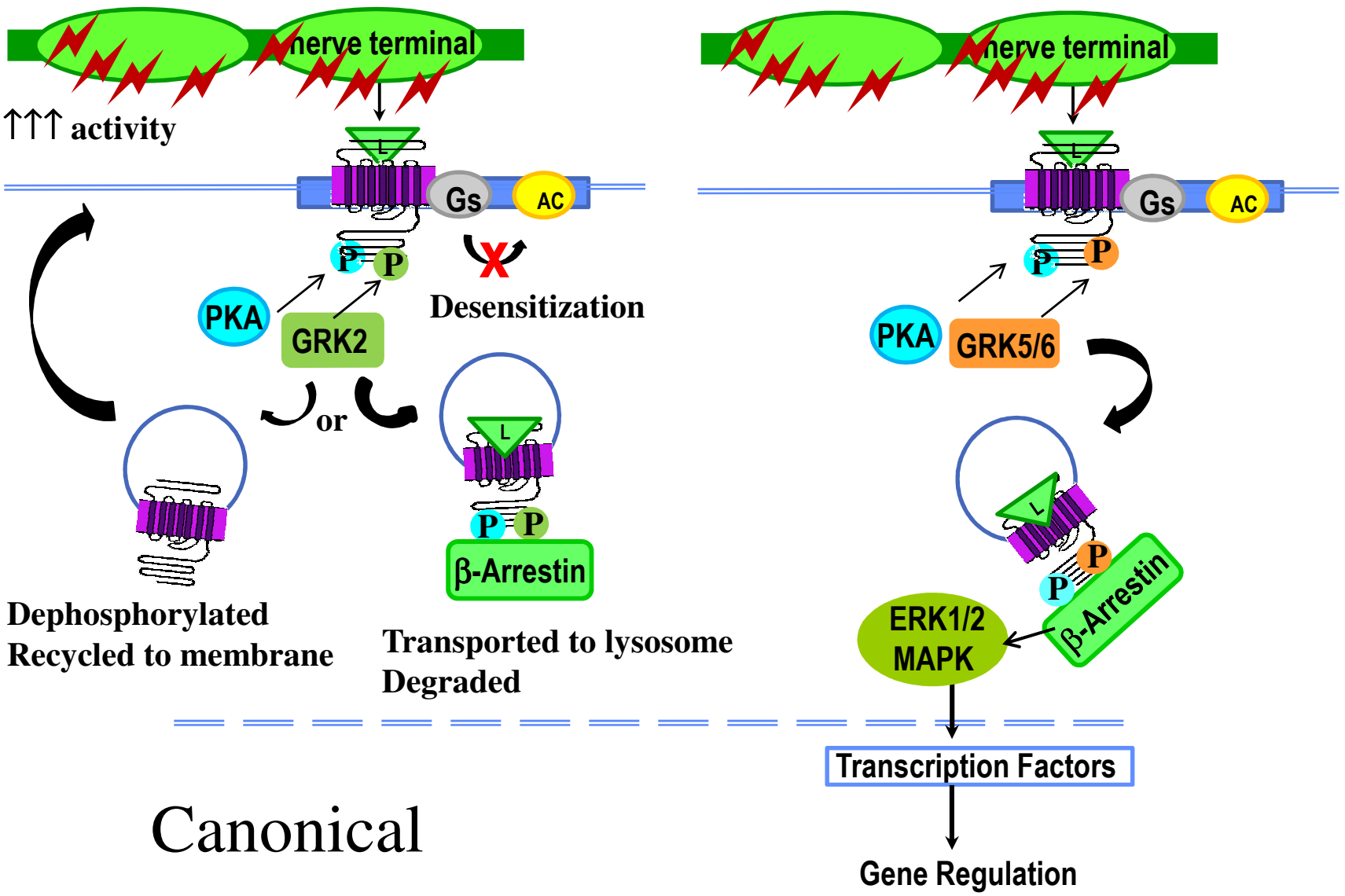
Altered Receptor Signaling in the DLN?



Canonical