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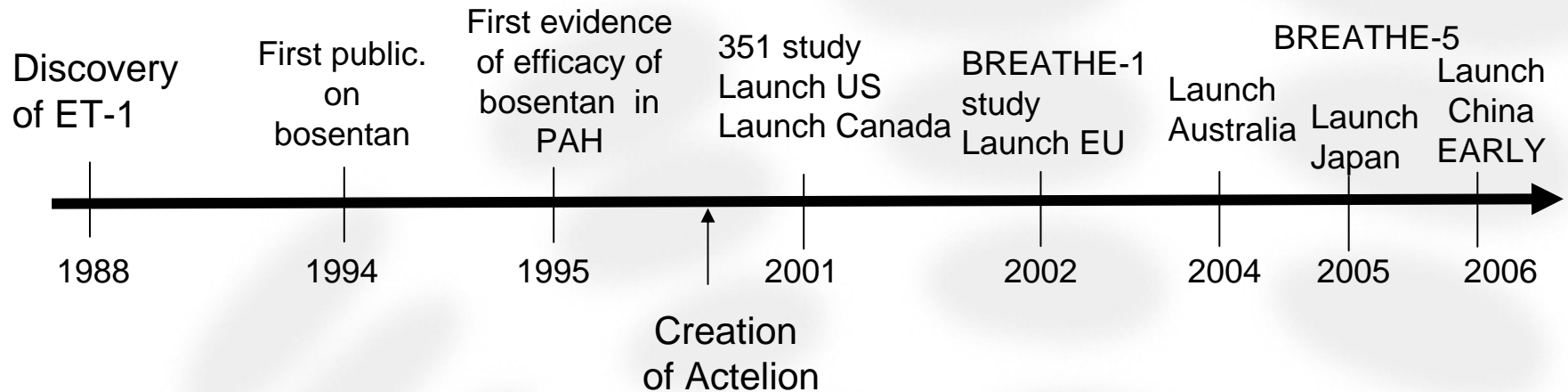
# The story of endothelin antagonists

Martine Clozel

Actelion Pharmaceuticals Ltd  
Allschwil, Switzerland



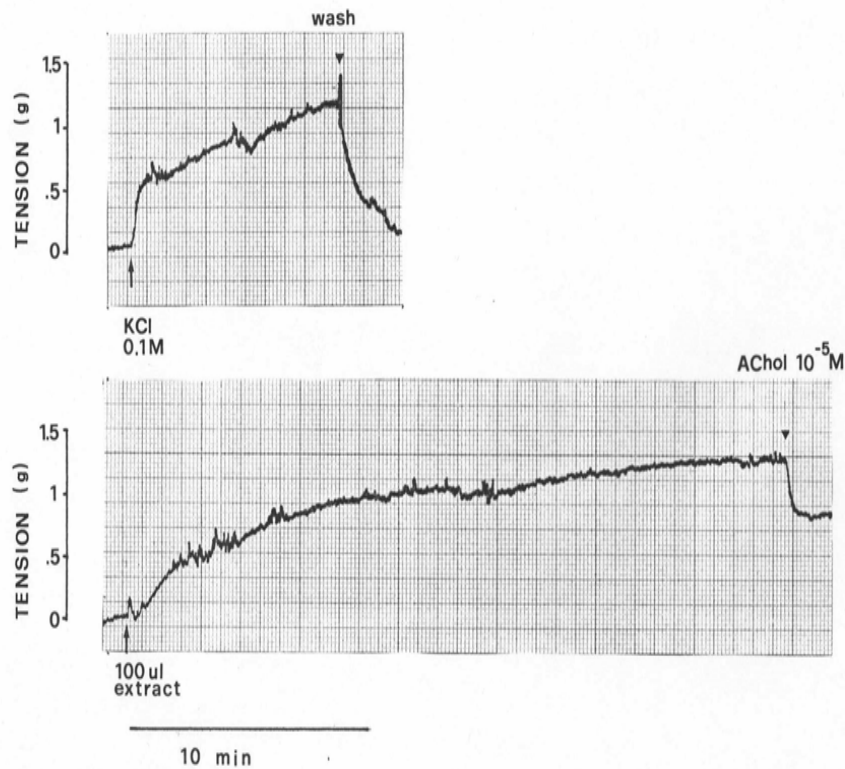
# Development of Tracleer® (bosentan)



18 years' research in ET and ET receptor antagonists,  
more than 130 manuscripts published by our group



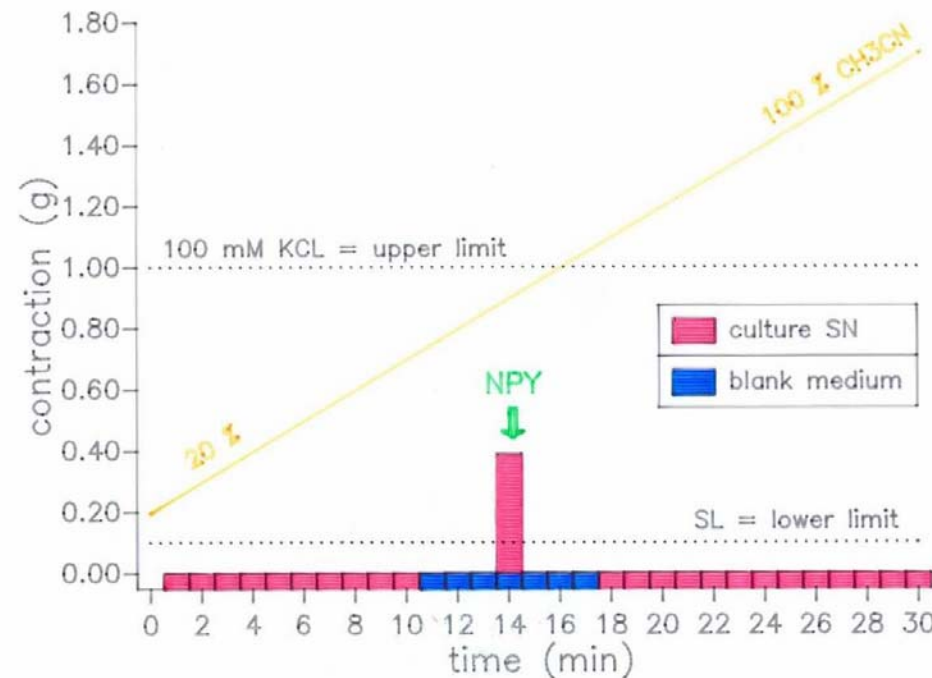
# Conditioned Medium from Human Endothelial Cells induces constrictions of rat aorta





# HLPC Fractionation of Conditioned Medium from Human Endothelial Cells

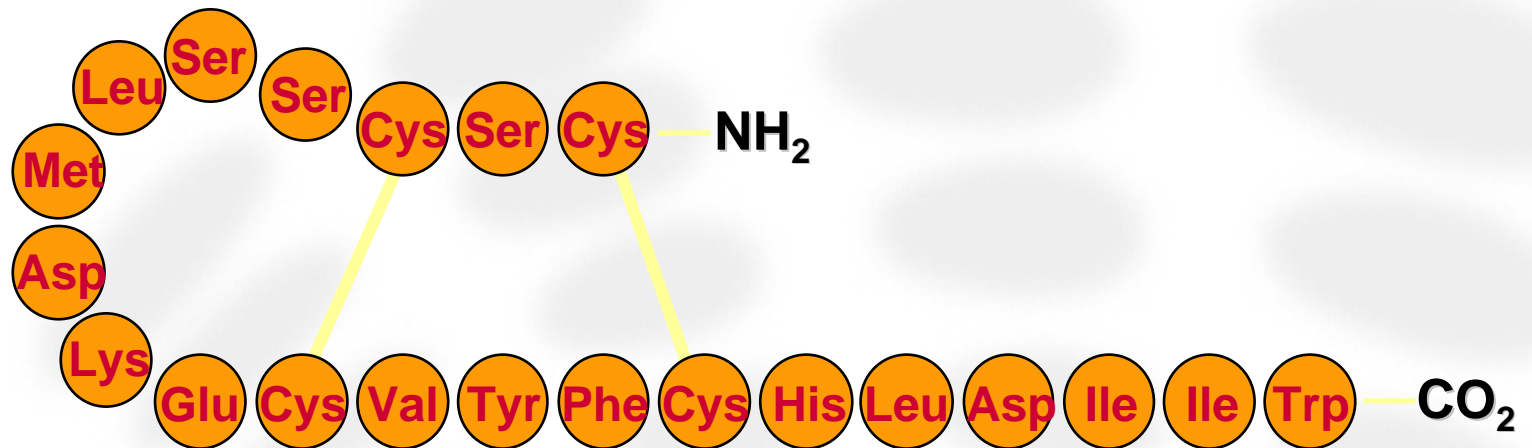
## RP-HPLC FRACTIONATION (2) OF UPCONCENTRATED MEDIUM



— concentrate (300 ul in KH - buffer) from 100 ml medium injected  
— fractions (1ml) in 500 ul KH - buffer --> 100 ul assayed  
(--> 20 ml medium)



# Endothelin-1





## Israeli mole viper



*(Atractaspis engaddensis)*

Kloog Y., et al., *Science*, 1988



# The first year after the discovery of ET-1

## Human Cultured Endothelial Cells Do Secrete Endothelin-1

Martine Clozel and Walter Fischli

*Journal of Cardiovascular Pharmacology*  
13(Suppl. 5):S229–S231 © 1989 Raven Press, Ltd., New York

SPECIFIC RECEPTORS FOR ENDOTHELIN ON MEMBRANES FROM  
HUMAN PLACENTA. CHARACTERIZATION AND USE IN A BINDING ASSAY.

Walter Fischli\*, Martine Clozel and Claire Guilly\*\*

*Life Sciences*, Vol. 44, pp. 1429–1436

## Specific Binding of Endothelin on Human Vascular Smooth Muscle Cells in Culture

Martine Clozel, Walter Fischli, and Claire Guilly

*J. Clin. Invest.*

Volume 83, May 1989, 1758–1761

## Effects of Endothelin on Regional Blood Flows in Squirrel Monkeys

MARTINE CLOZEL and JEAN-PAUL CLOZEL

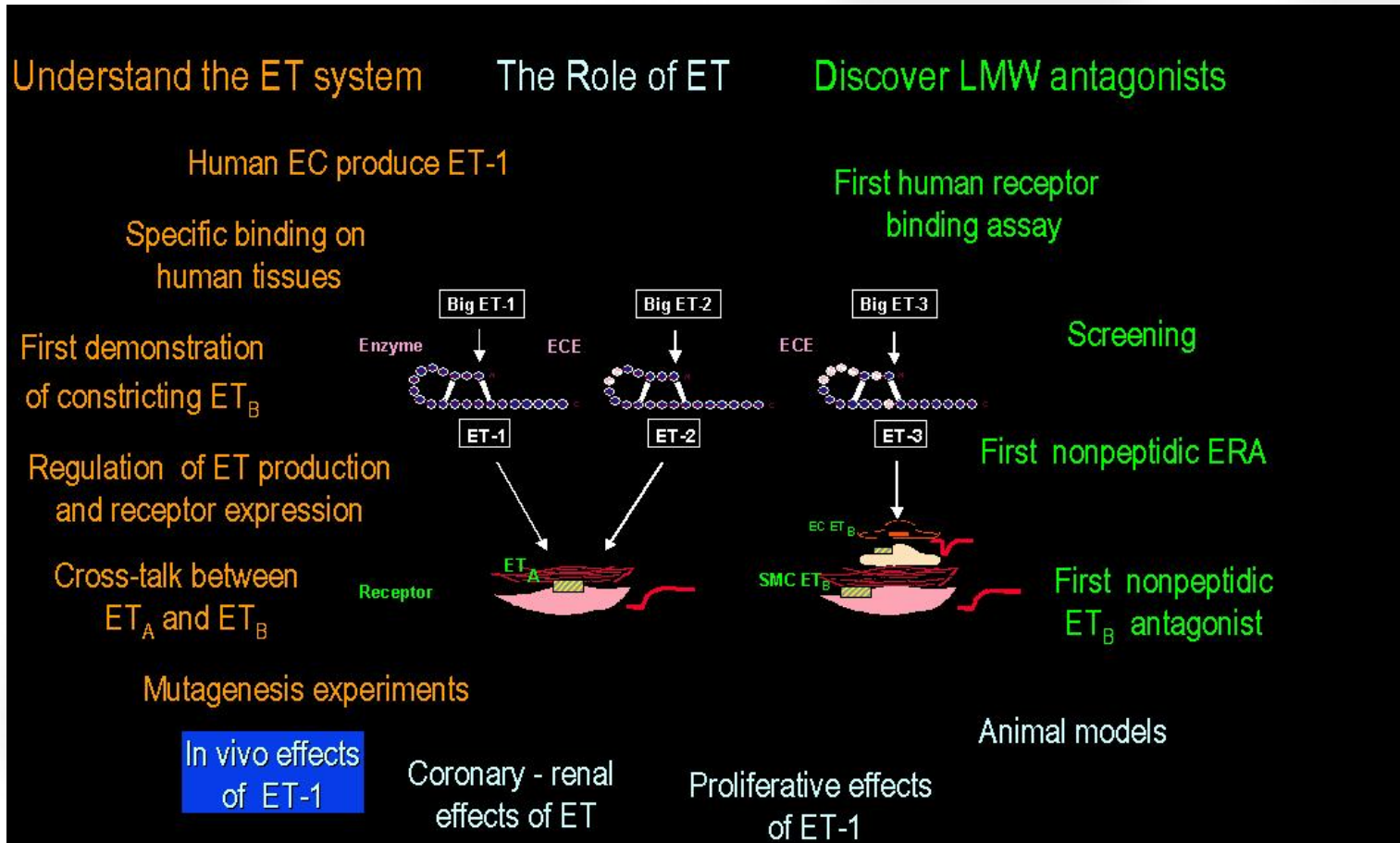
THE JOURNAL OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

## Endothelin sensitivity and receptor binding in the aorta of spontaneously hypertensive rats

Martine Clozel

*Journal of Hypertension* 1989, 7:913–917

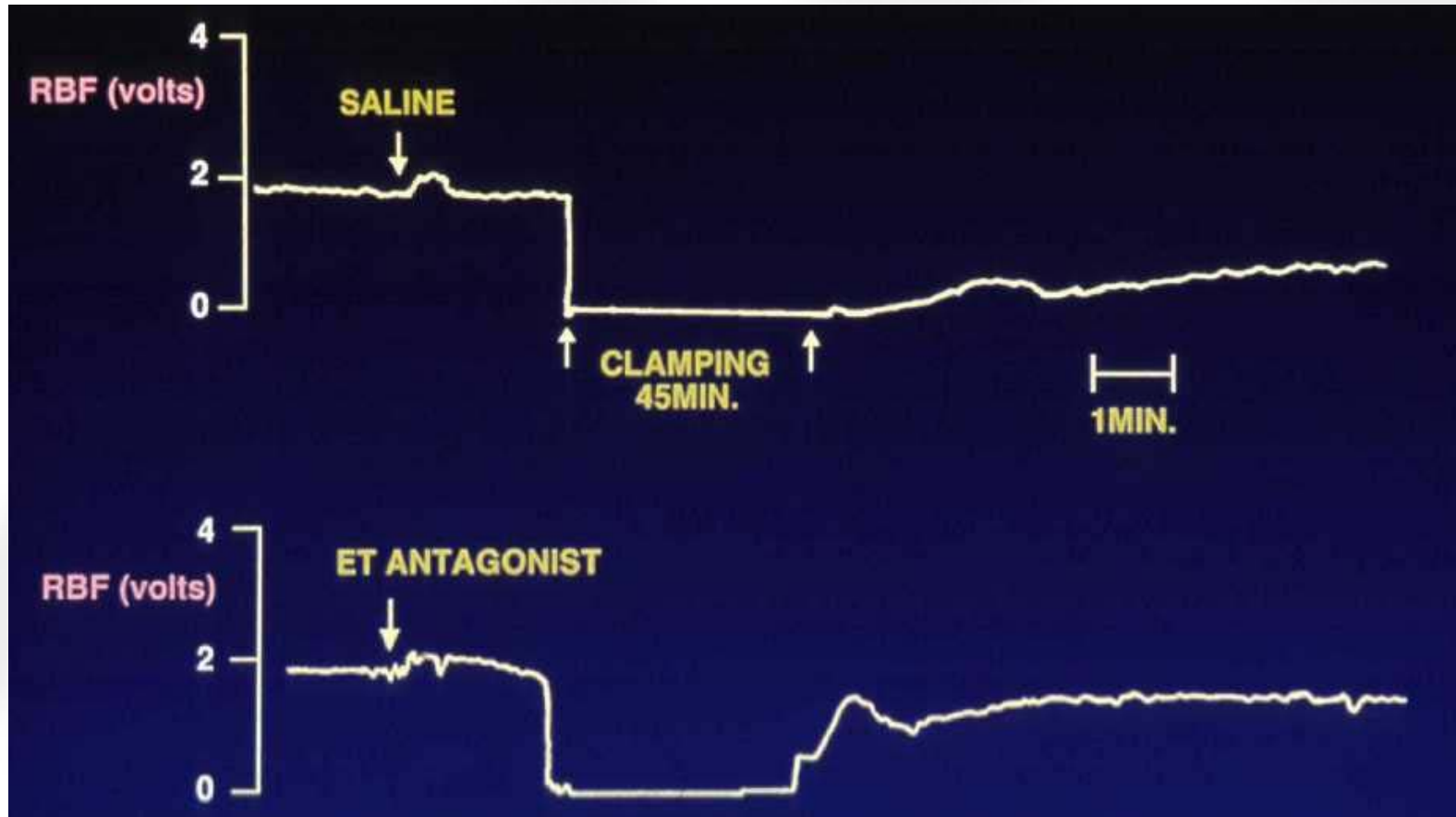
# Our contribution







## Effect of ERA on No-Reflow after Renal Ischemia in Rats



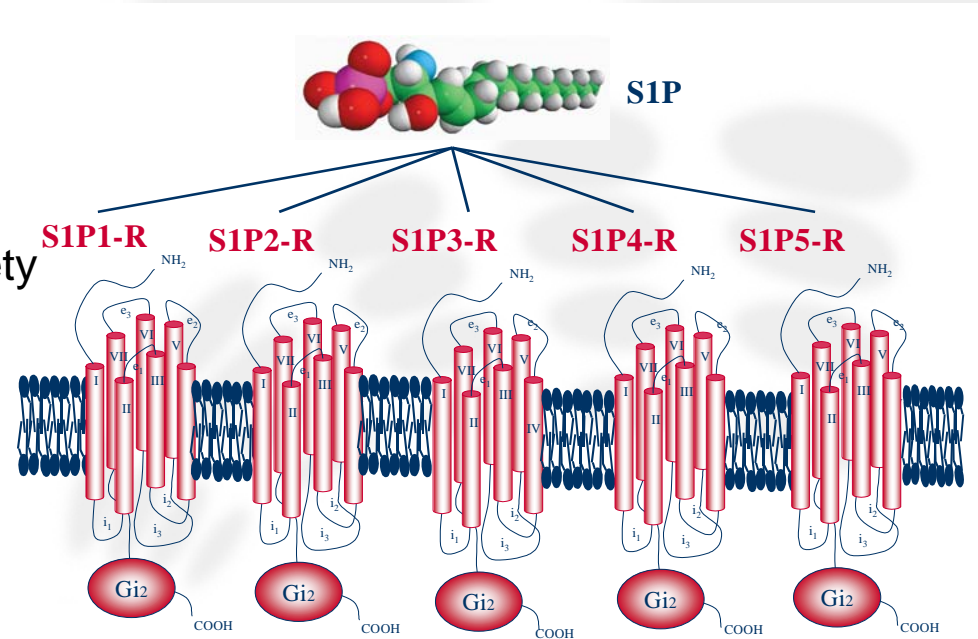


# **Selective Sphingosine-1 Phosphate Receptor-1 (S1P<sub>1</sub>) Agonists**

Novel cutting-EDG agents for controlled immunomodulation

# S1P receptors – A pleiotropic system

- S1P is a phospholipid released by platelets, mast and other cells
- S1P stimulates 5 GPC receptors S1P<sub>1,2,3,4,5</sub>
- The different receptors induce a variety of biological responses





# The project at Actelion

- Actelion started in 1999 to work on S1P<sub>1</sub> (then called Edg-1) because of its localization on the endothelium
- High-Throughput Screening yielded multiple hits
- Lead optimization program resulted in highly active agonists



# Mechanism of FTY720 discovered

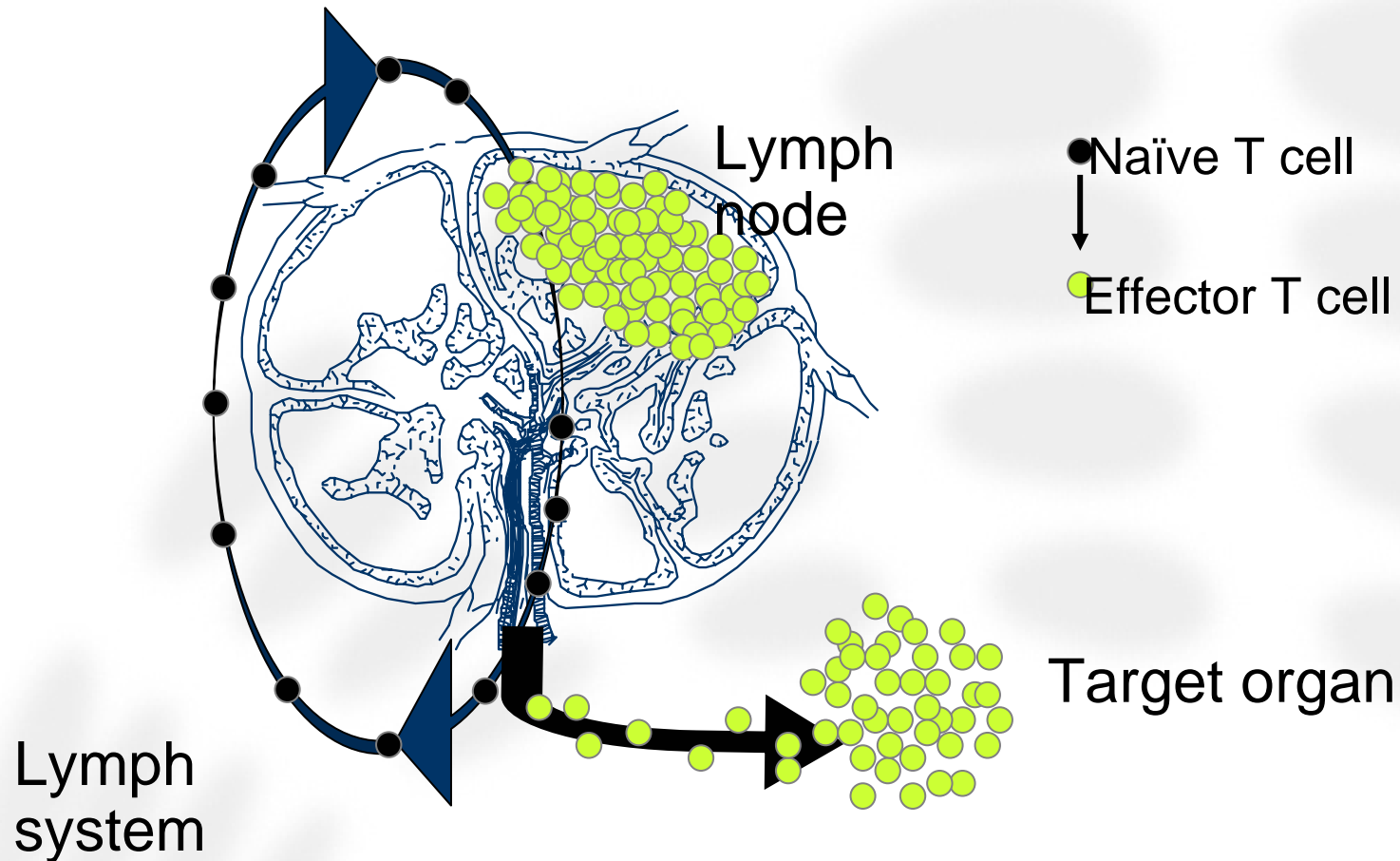
12 APRIL 2002 VOL 296 SCIENCE [www.sciencemag.org](http://www.sciencemag.org)

## Alteration of Lymphocyte Trafficking by Sphingosine-1-Phosphate Receptor Agonists

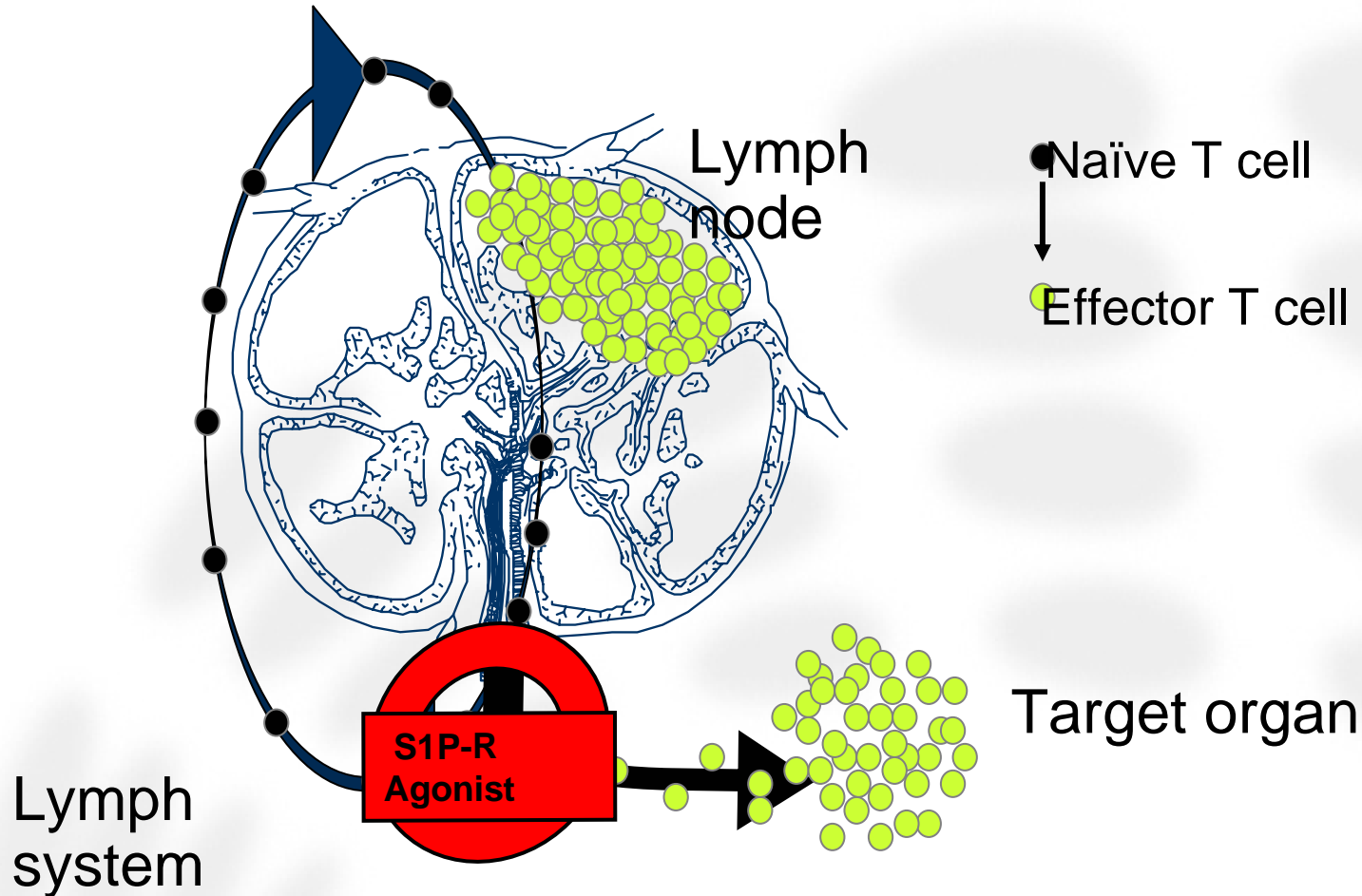
Suzanne Mandala,<sup>1</sup> Richard Hajdu,<sup>1</sup> James Bergstrom,<sup>1</sup>  
Elizabeth Quackenbush,<sup>2</sup> Jenny Xie,<sup>2</sup> James Milligan,<sup>1</sup>  
Rosemary Thornton,<sup>1</sup> Gan-Ju Shei,<sup>1</sup> Deborah Card,<sup>1</sup>  
CarolAnn Keohane,<sup>1</sup> Mark Rosenbach,<sup>1</sup> Jeffrey Hale,<sup>3</sup>  
Christopher L. Lynch,<sup>3</sup> Kathleen Rupprecht,<sup>3</sup> William Parsons,<sup>3</sup>  
Hugh Rosen<sup>1\*</sup>

Blood lymphocyte numbers, essential for the development of efficient immune responses, are maintained by recirculation through secondary lymphoid organs. We show that lymphocyte trafficking is altered by the lysosphospholipid sphingosine-

## Mechanism of Action

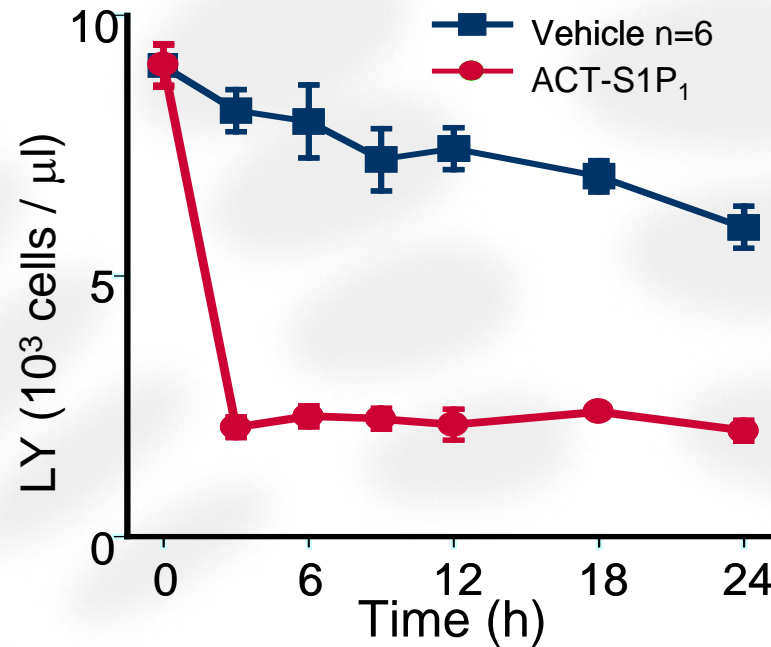


## Mechanism of Action





# Effect of the selective agonist ACT-S1P<sub>1</sub> on lymphocyte count in rats

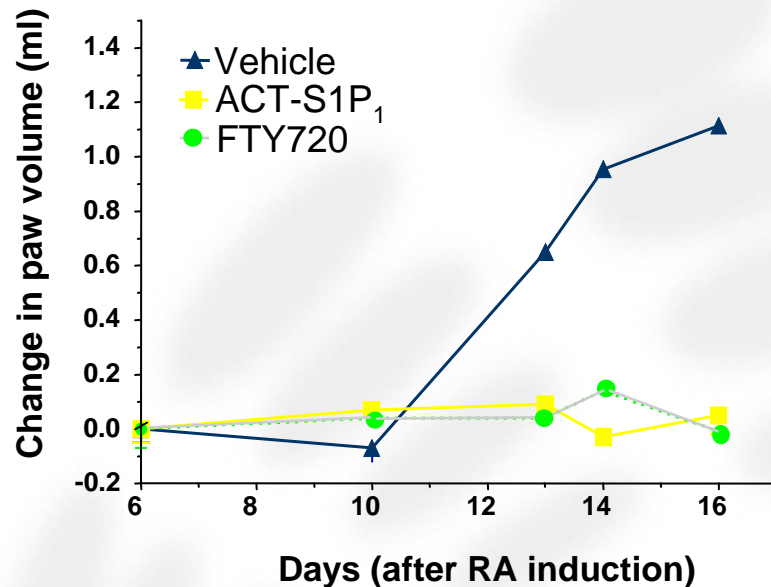


- Substantial decrease of circulating lymphocytes
- Rapid onset of action

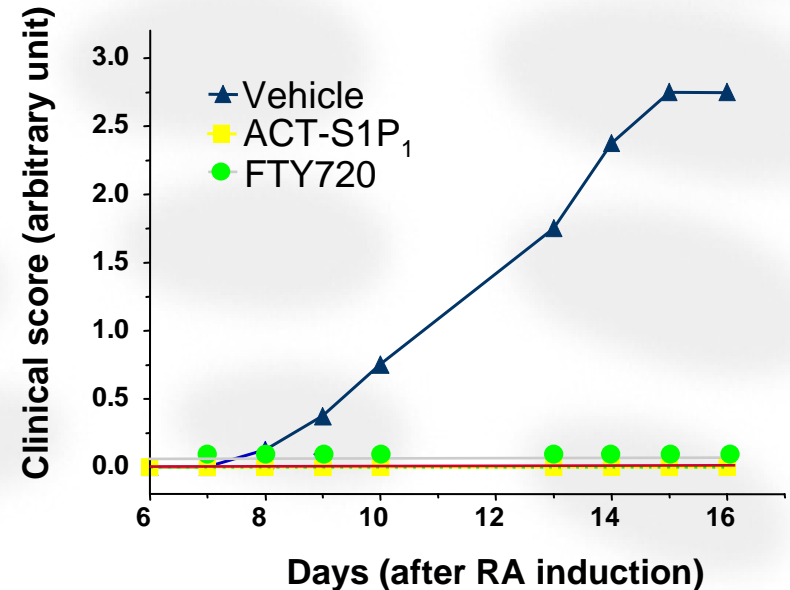


# The selective agonist ACT-S1P<sub>1</sub> is as efficacious as FTY720

## Paw volume



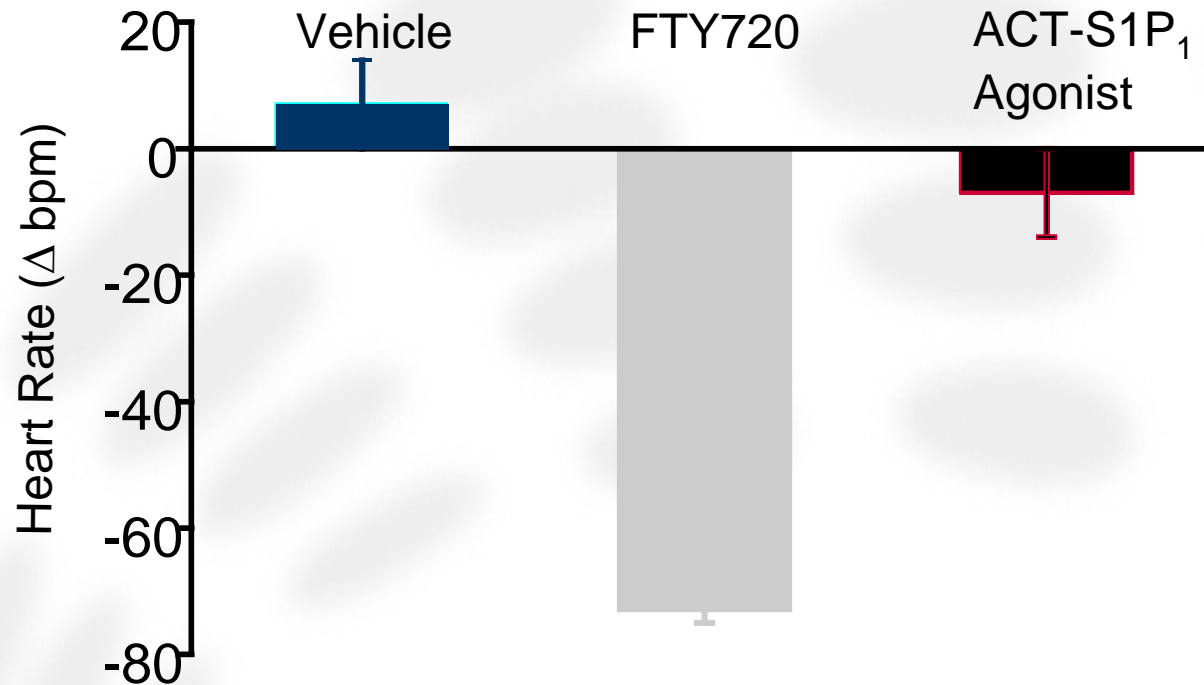
## Clinical score



ACT-S1P<sub>1</sub> agonist prevents arthritis in rats

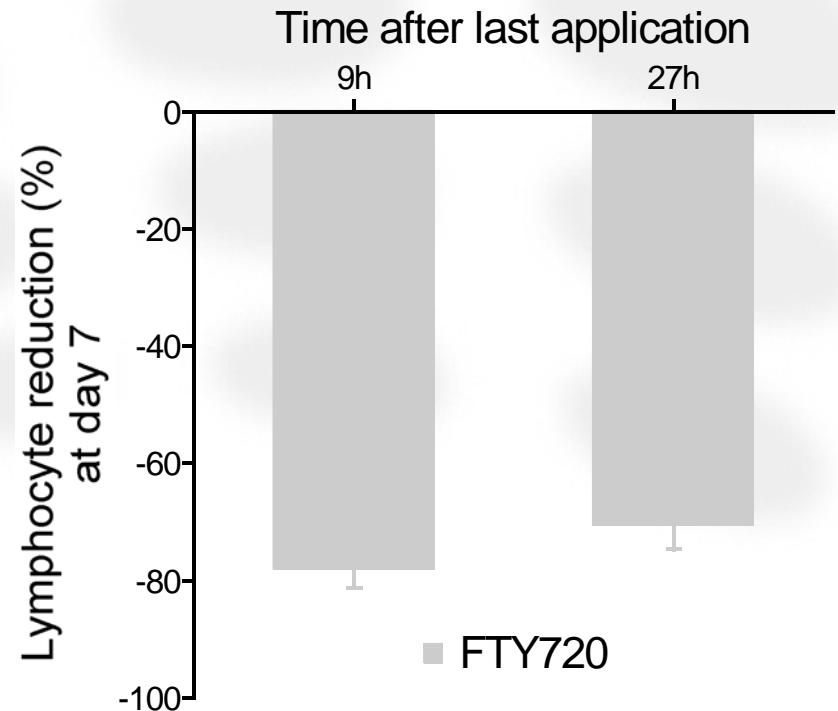
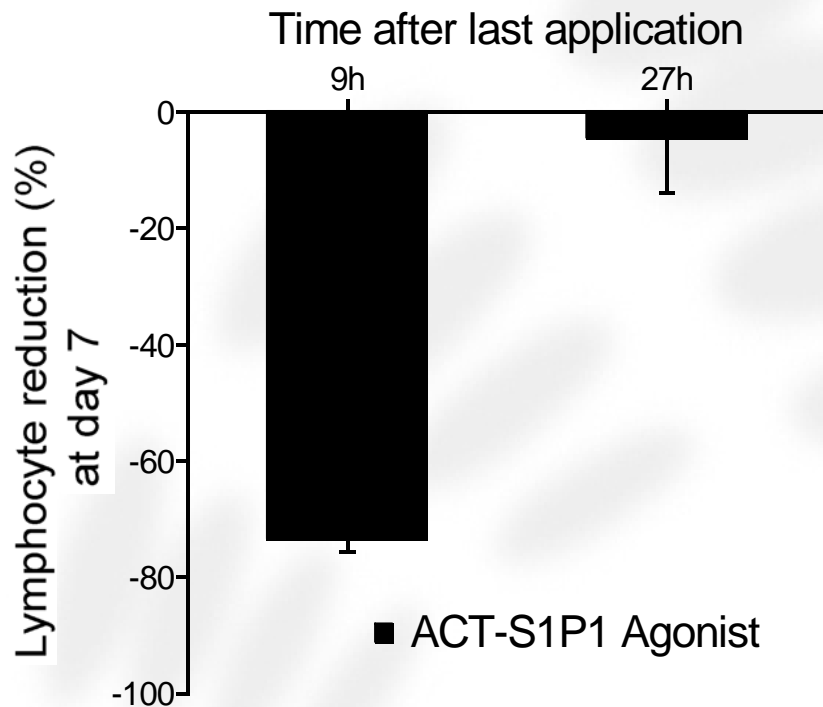


# The selective agonist ACT-S1P<sub>1</sub> does not induce bradycardia in rats



# The effect of ACT-S1P<sub>1</sub> is rapidly reversible

## 7-day study in rat





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# The Orexin Receptor Antagonist



## Orexin reduces REM (rapid eye movement) sleep

REM sleep is virtually abolished by orexin-A in the rat

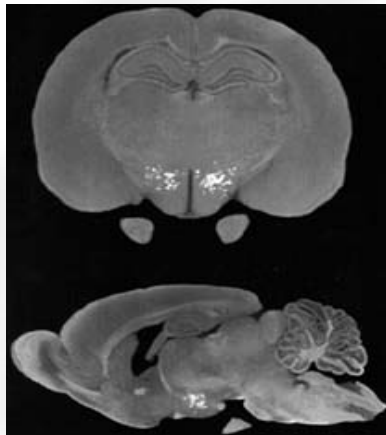
	Vehicle	Orexin A (30 $\mu\text{g}/\text{rat}$ , i.c.v.)	Change (%)
Time in REM-sleep (% total sleep time)	10.7	1.4	- 87 %

Adapted from *Piper et al., 2000*



# Orexins

- Orexins were discovered in 1998  
as peptides of appetite control  
*De Lecea et al., 1998, Sakurai et al., 1998*  
they are actually peptides of „vigilance“
- Orexin deficiency leads to hypersomnolence  
*Lin et al., 1999, Chemelli et al., 1999*



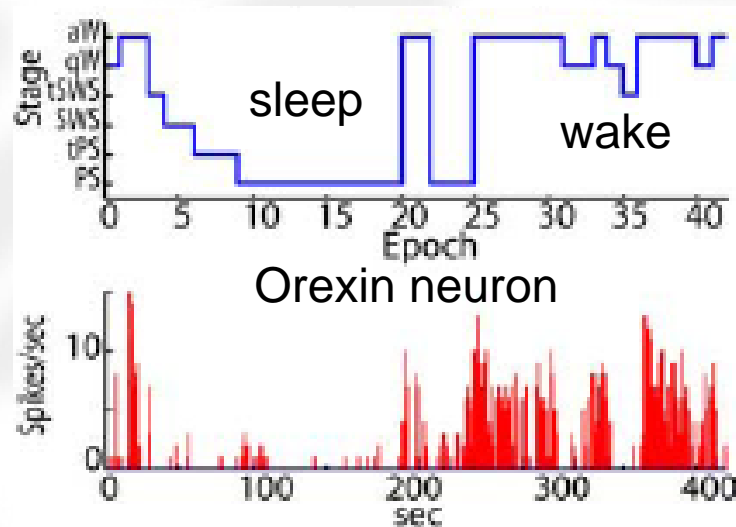
Orexin production in the lateral hypothalamus



# Orexin neurons keep us awake and prevent REM sleep

Orexin neurons are

- maximally active during active waking
- virtually silent during sleep and REM sleep



*Lee et al., 2005*



## A specific and potent orexin receptor antagonist

- Selectivity in in-vitro biochemical assays:

Receptor or enzyme	IC <sub>50</sub> (nM)
<b>Orexin</b>	<b>&lt; 10</b>
Melatonin	> 5'000
Benzodiazepine	>10'000
GABA-A, B	>10'000
Opiate- $\mu, \delta, \kappa$	>10'000
Histamine-1,2,3	>10'000
+ 85 other assays	>10000

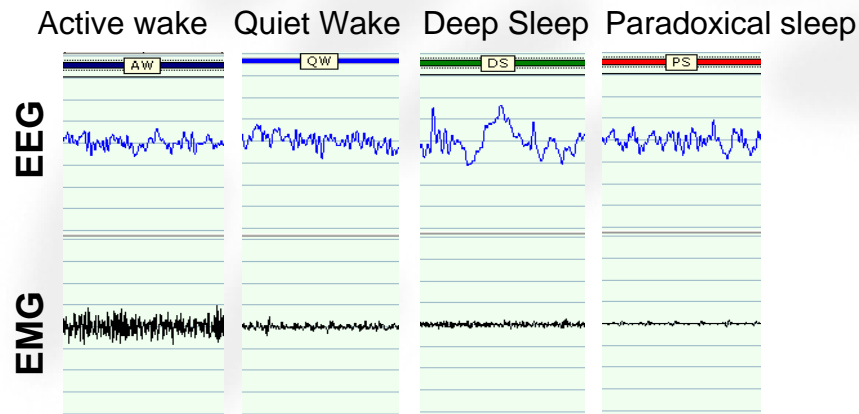
- Good oral bioavailability
- Excellent brain penetration



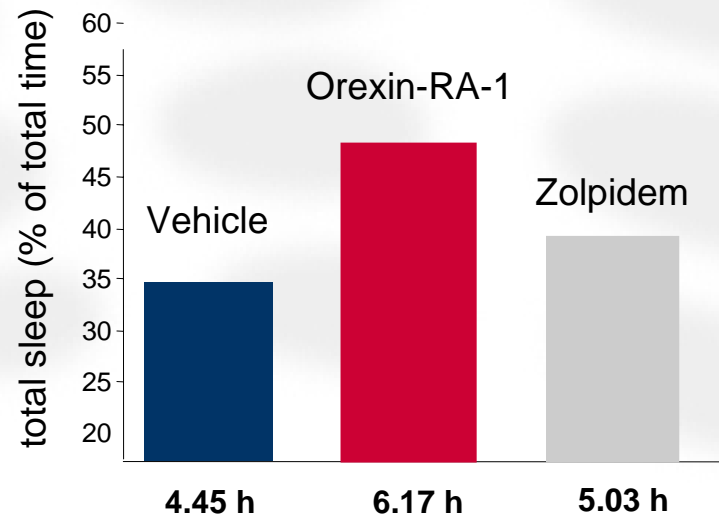


# Orexin-RA-1 increases sleeping time

Sleep EEG recorded in the rat using radiotelemetric transmitters

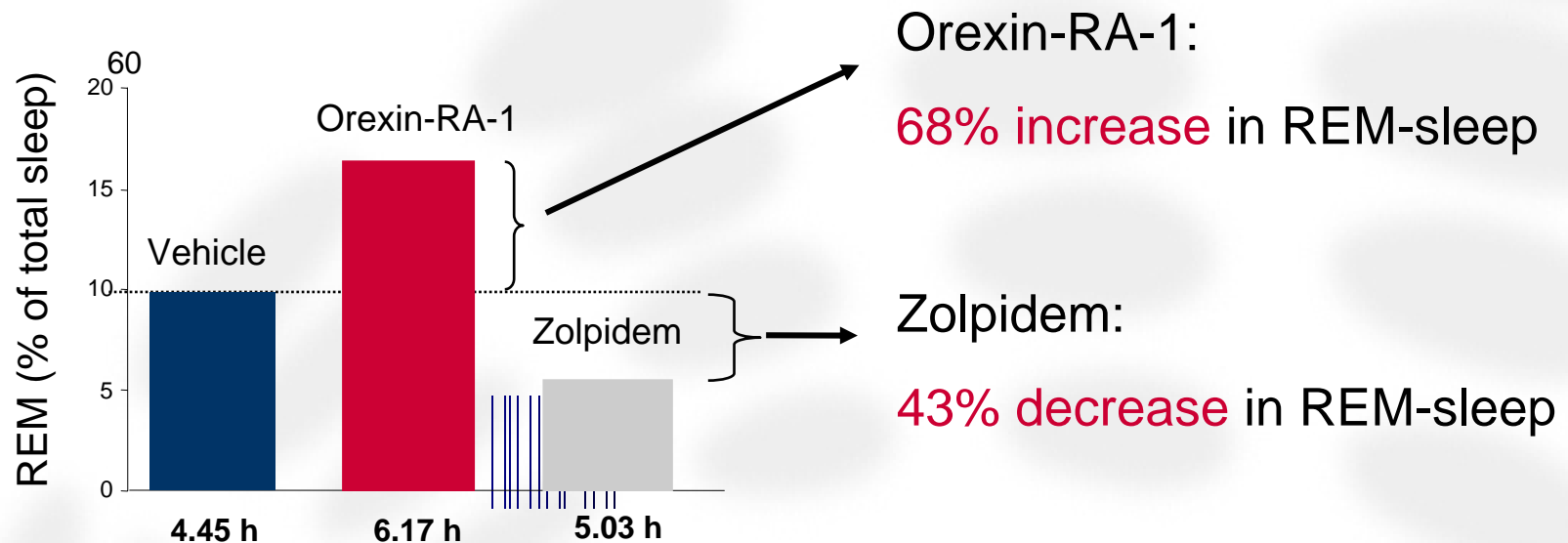


## Total sleep time





# Orexin-RA-1 increases REM-sleep

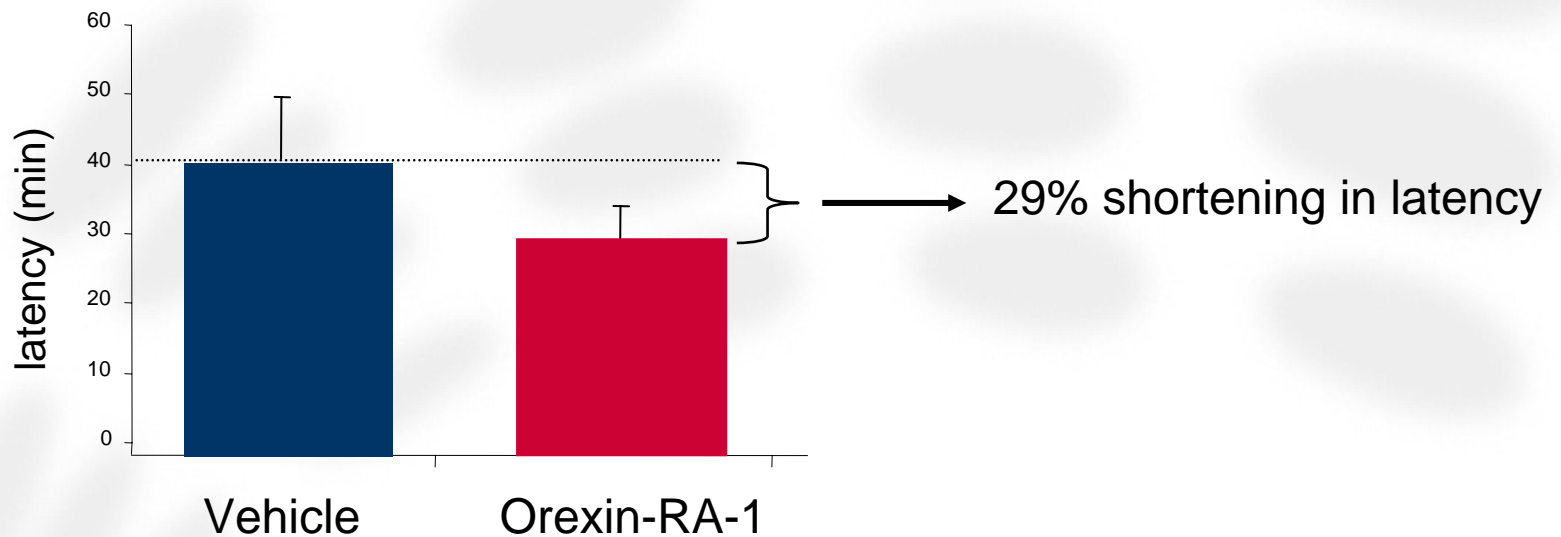


REM stage - and accompanying dream sequences -  
is linked to memory consolidation



# Orexin-RA-1 accelerates time to fall asleep

## Latency to first episode of persistent sleep



# Orexin-RA-1 does not cause motor impairment

