Department of Molecular Biosciences
University of Graz
Graz, Austria

T. Hanhoff, C. Wolfrum (Münster, Germany)

Y. Owada, H. Kondo (Sendai, Japan)

M. Pelsers,J. Glatz, J. de Kruijk, A. Honig, P. Menheere (Maastricht, NL)

W. Rudziński (Bialystok, Poland)

The fatty acid binding proteins (FABPs) of brain and their potential as neurobiochemical markers of brain damage

fritz.spener@uni-graz.at

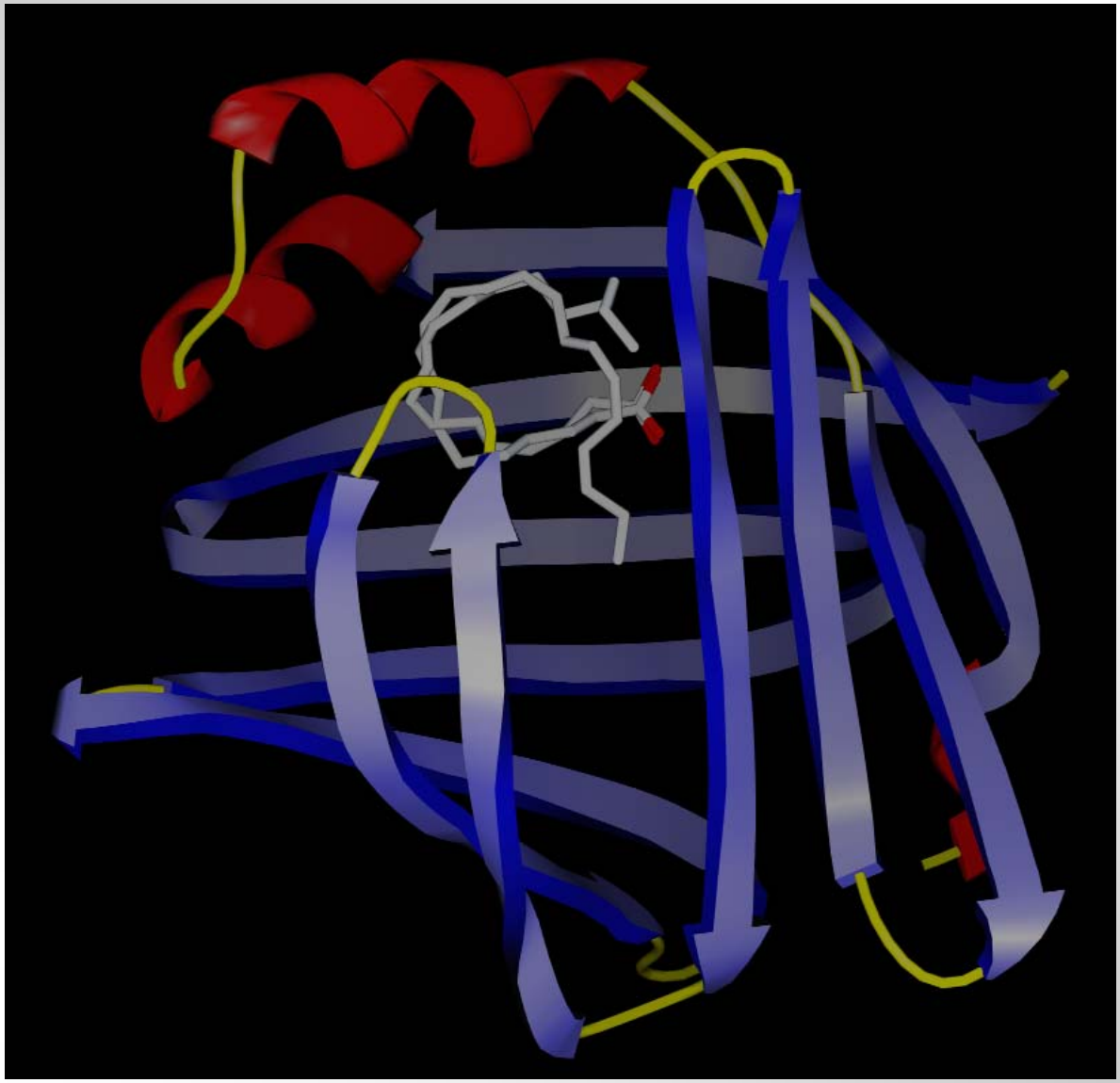
Lipids and Brain, Journées Chevreul, Paris, France, 13-14 March 2007

Overview

- Introduction into FABPs
- FABP function in mouse brain
- Tissue distribution of FABPs in human brain
- FABPs as serum markers for brain injury in humans

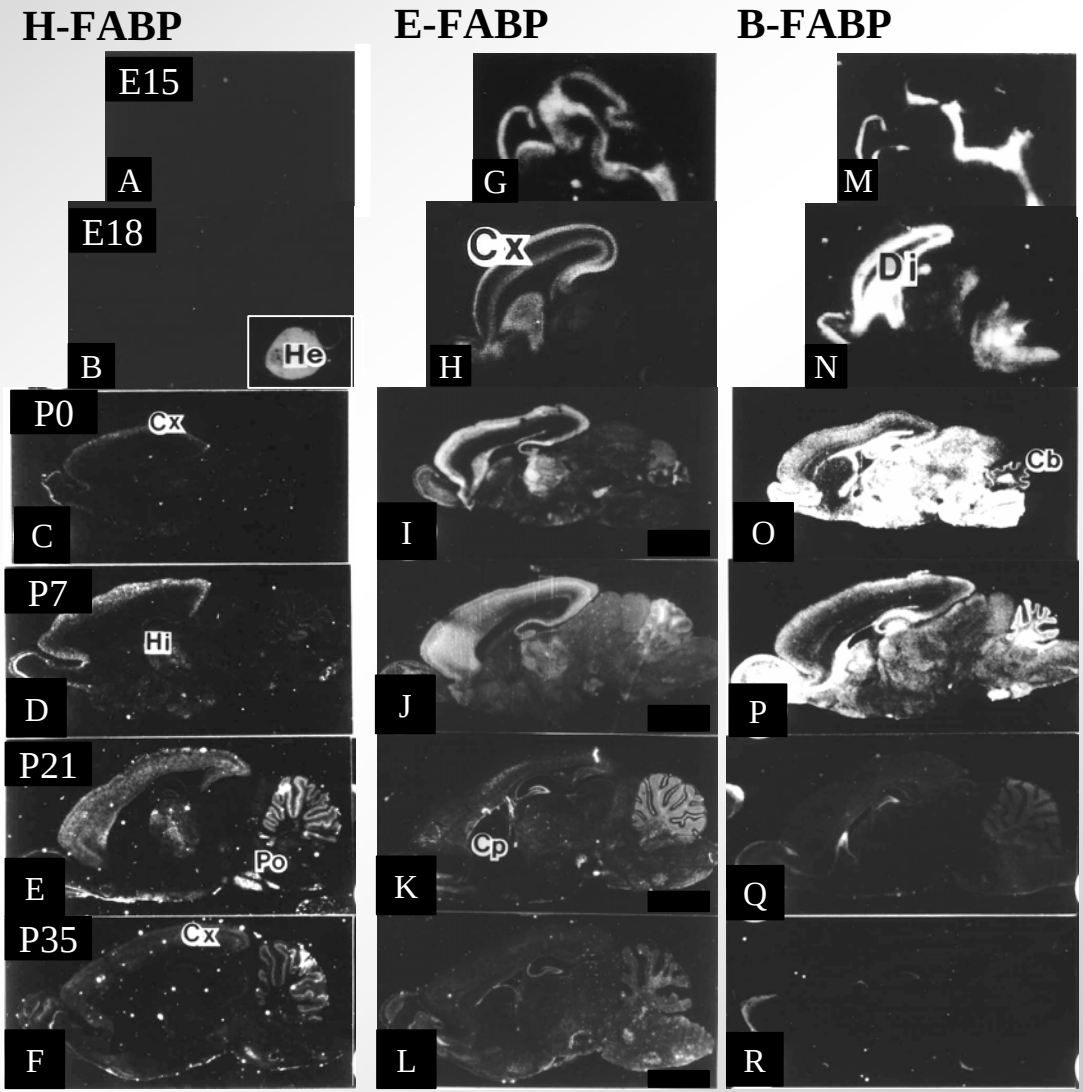
Fatty acid binding proteins

- 15 kDa paralogs (9 types in mammals),
- Involved in intracellular fatty acid transport and signaling
- Spatio/temporal differentially expressed in various tissue cells
- Subcellular localization in cytoplasm and nucleus
- Expression in brain:
  - Brain-type FABP (B-FABP) in astrocytes
  - Heart-type FABP (H-FABP) in neurons
  - Epidermal-type FAPP (E-FABP) in astrocytes and neurons

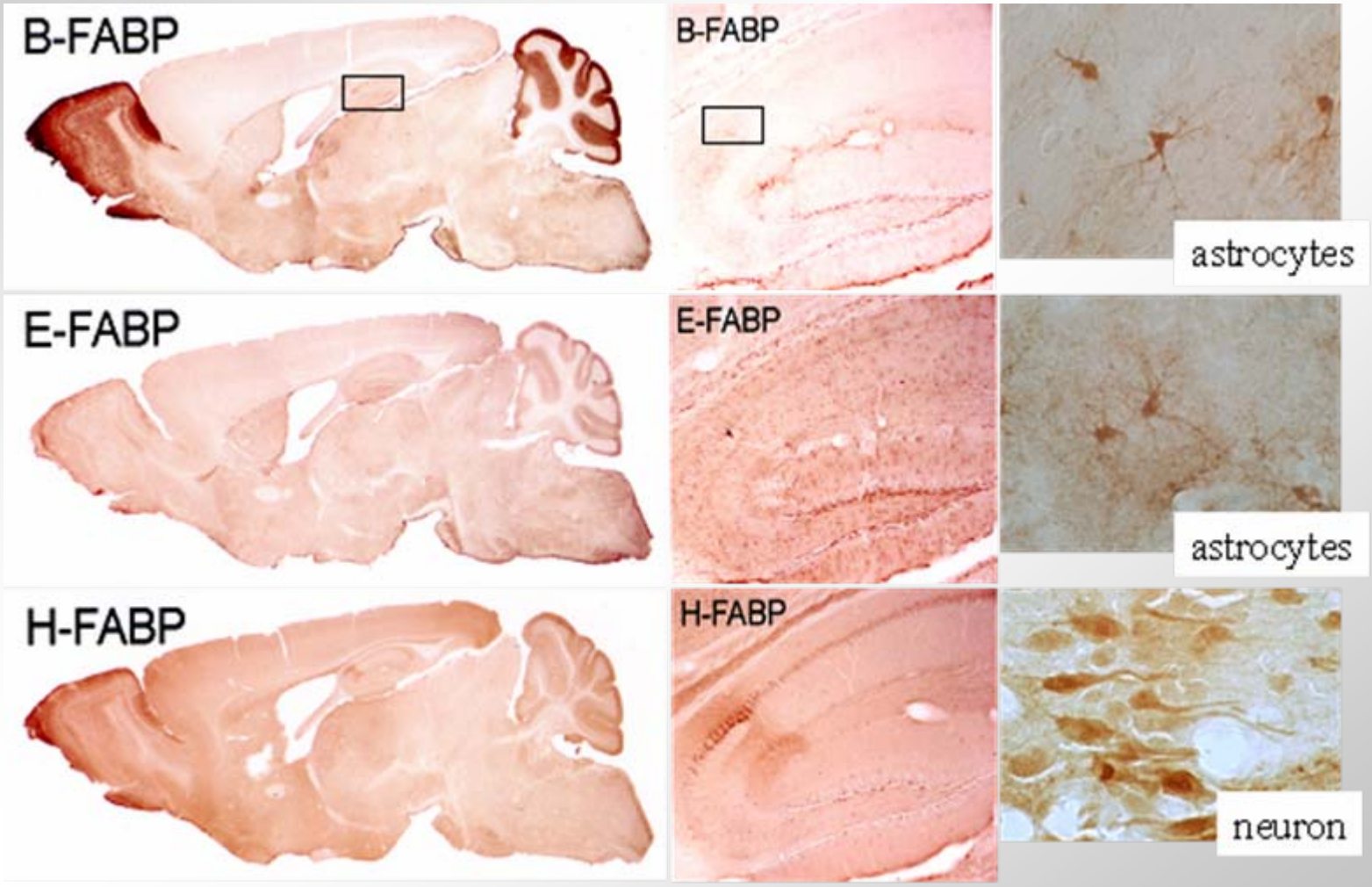


Balendrian et al. (2000) J. Biol. Chem. 275, 27045-27054

Spatio-temporal expression of FABP mRNAs during mouse brain development

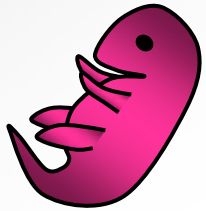


FABPs expressed in the mature mouse brain

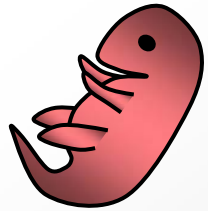


Absence of B-FABP protein and mRNA expression in the brain of male B-FABP null mice

ventricular zone at embryonic day18

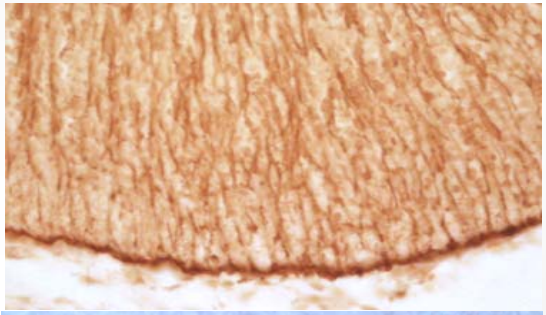


wild

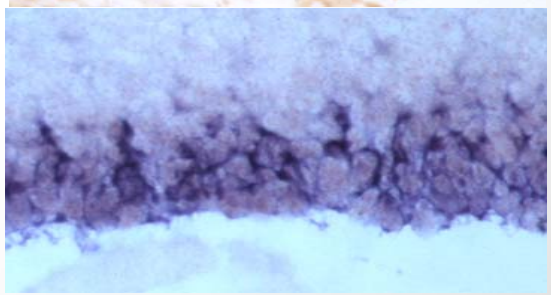


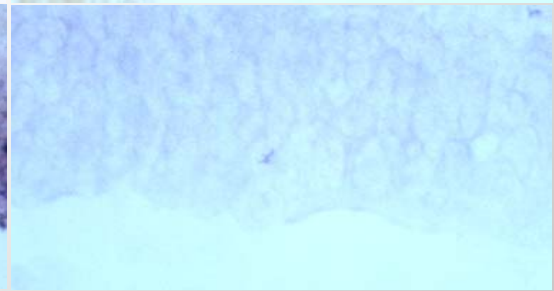
B-FABP null

B-FABP protein



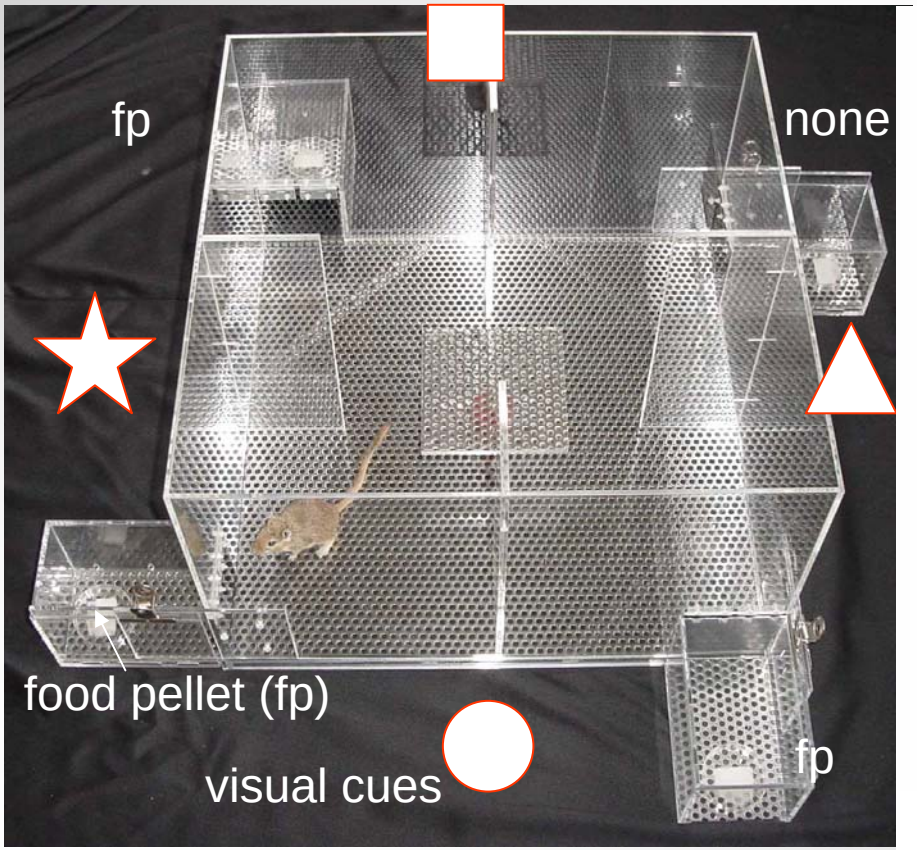
B-FABP mRNA

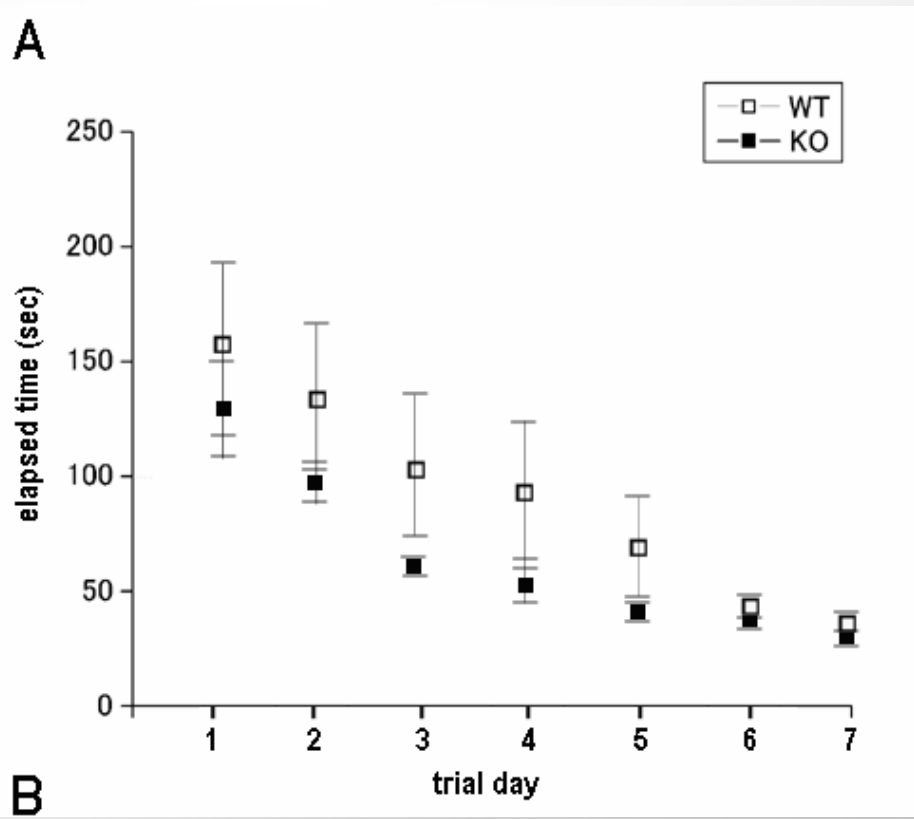




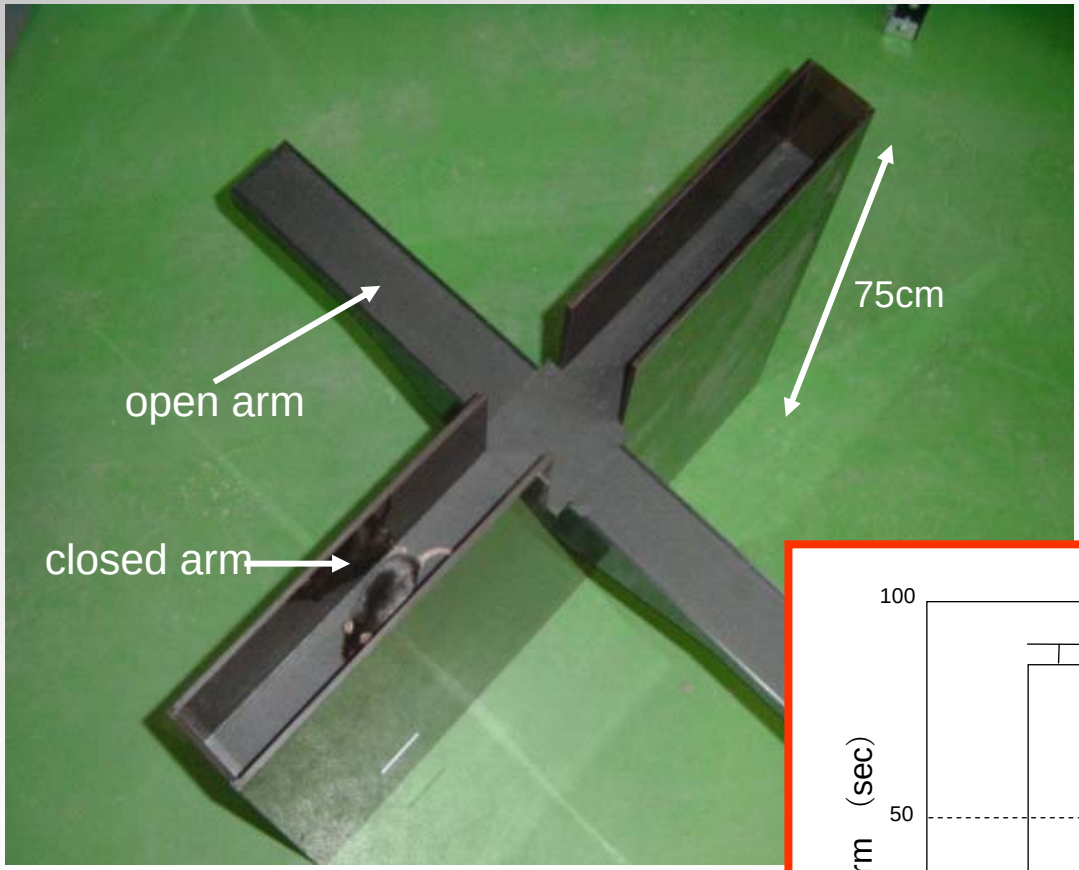
Normal spatial learning and memory in B-FABP null mice

food pellet-taking test

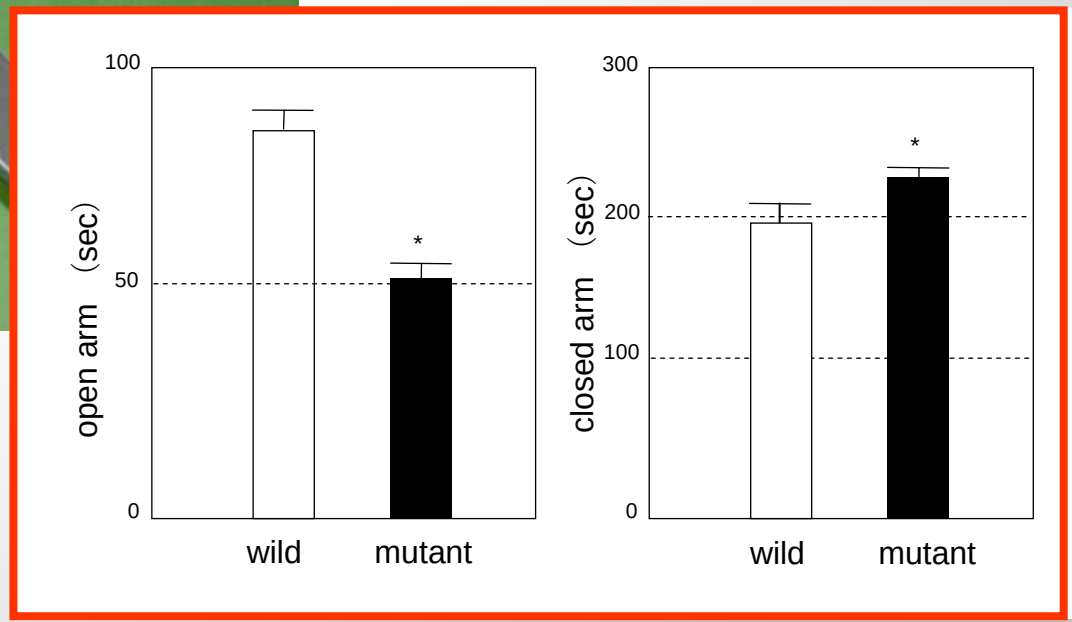




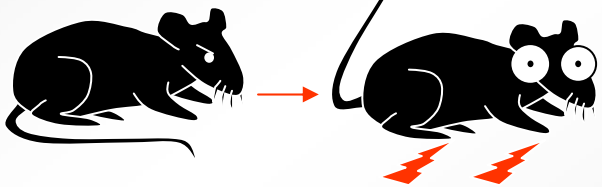
Increased anxiety in B-FABP null mice



elevated plus maze test

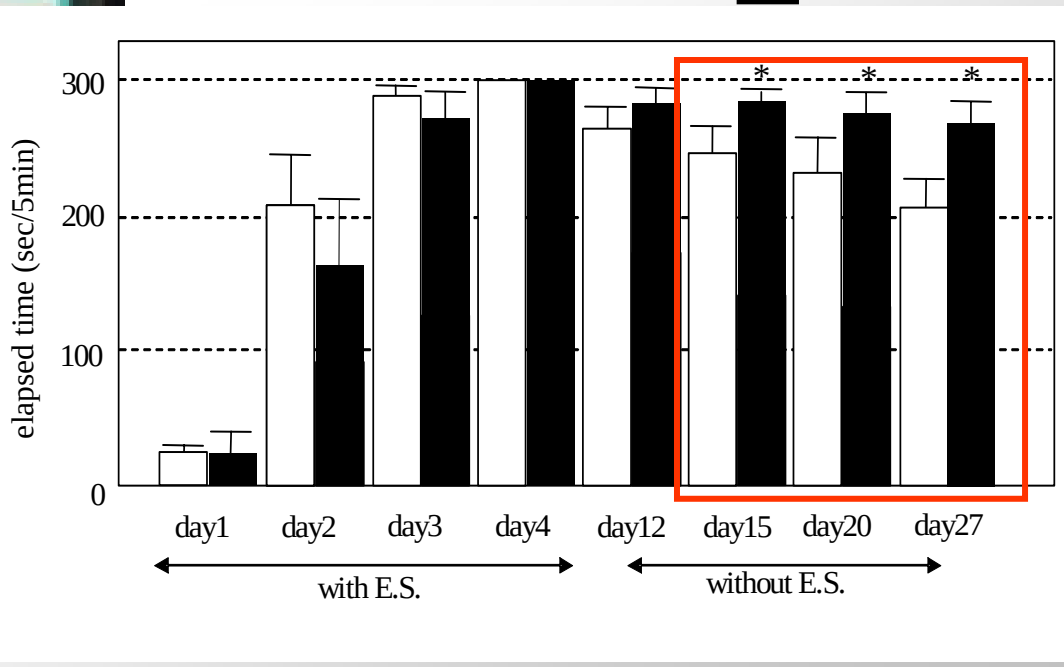


Prolonged fear memory in B-FABP null mice



electrical foot shock (E.S)

passive avoidance test



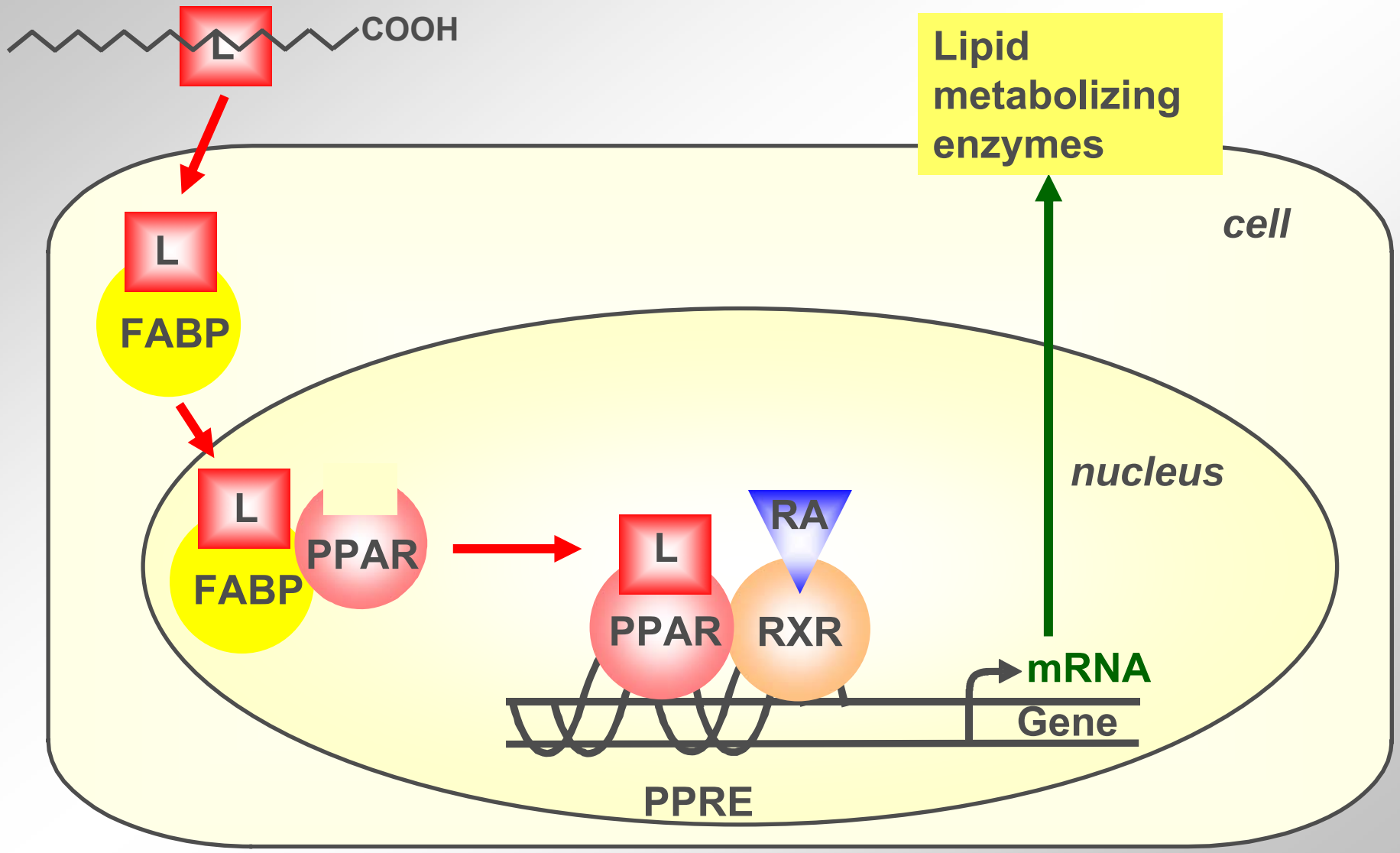
Very long-chain fatty acids in total phospholipids in brain from male wild-type and B-FABP null mice

	Brain at P0		Brain at P70	
Fatty acid	wild-type (%)	null (%)	wild-type (%)	null (%)
AA (20:4 n-6)	10.37 ± 0.18	10.76 ± 0.15*	9.00 ± 0.53	8.97 ± 0.24
DHA (22:6 n-3)	10.86 ± 0.23	10.33 ± 0.18*	15.93 ± 0.66	15.89 ± 0.67

Data are means ± SD (n=5); * $P < 0.05$ (Student's *t*-test).

Owada et al. (2006) Eur. J. Neurosci. 24, 175-187

The signaling path of fatty acids

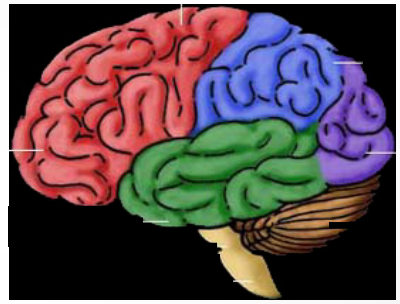


Wolfrum et al. (2001) Proc. Natl. Acad. Sci. USA 98, 2323-2328

Introduction into tissue injury detection

- Tissue injury (ischemia/reperfusion, rejection, trauma inflammation) leads to release of cytoplasmic proteins into the blood

- Early detection of these proteins contributes to early diagnosis and improved clinical treatment



The brain

- Brain injury (ischemia, trauma) difficult to detect in blood due to the blood-brain barrier

- Currently used marker proteins:
  - S100B, 21 kDa, astrocytes
  - MBP, 18.5 kDa, myelin
  - GFAP, 50 kDa, astrocytes
  - NSE, 90 kDa, neurons

Aims of study

I. Investigation of the tissue distribution of B- and H-FABP in the human brain

II. Evaluation of both proteins as serum markers for mild traumatic brain injury, electro-convulsive therapy and cerebro-vascular accidents compared with S100B and Neuron Specific Enolase (NSE)

Pelsers et al.(2004) Clin. Chem. 50, 1568-1575
Wunderlich et al. (2005) J. Neurol. 252, 718-724

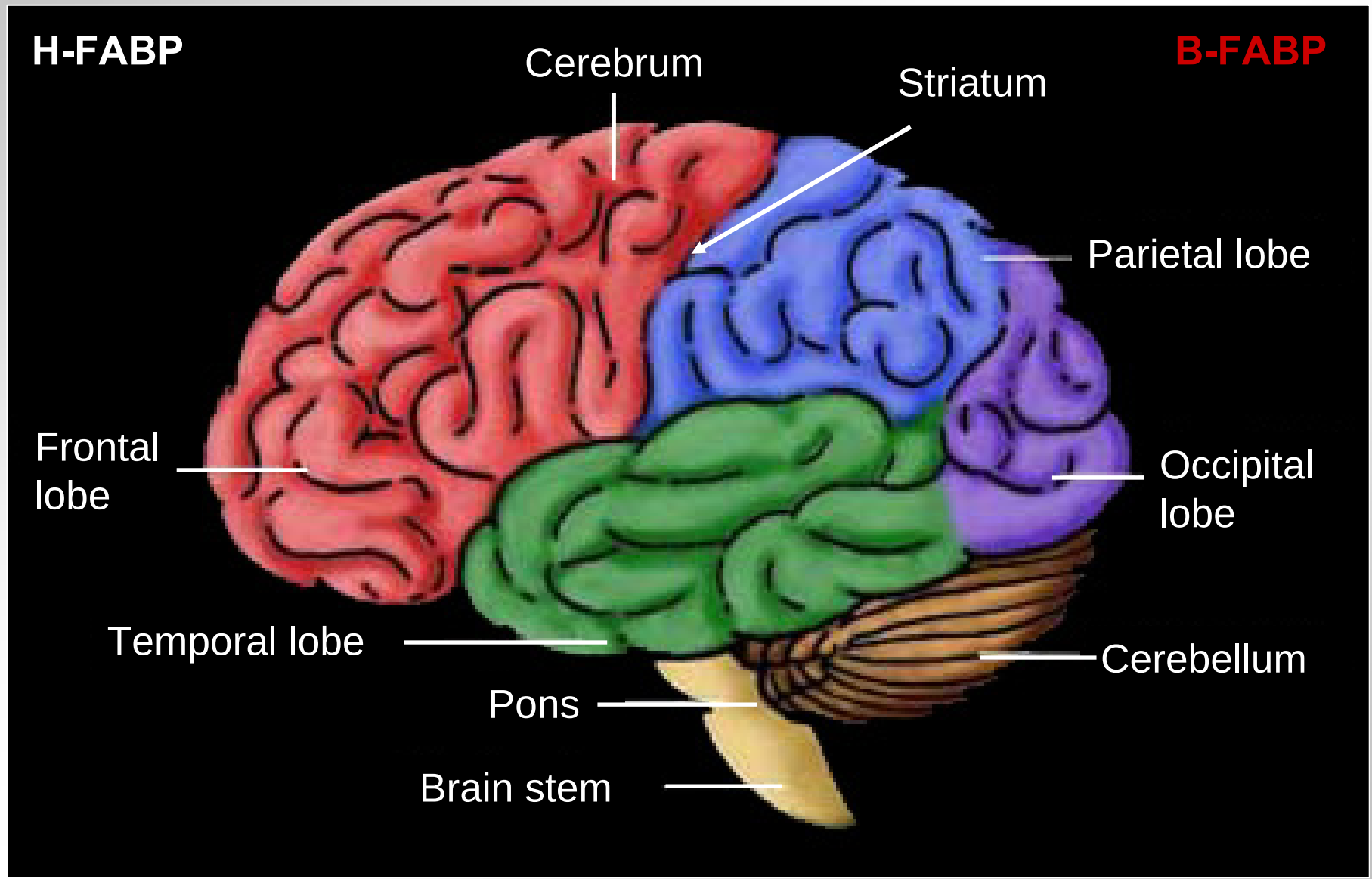
Methods

- Homogenization, ultrasonic treatment and centrifugation of tissue samples
- ELISAs for **H-FABP, B-FABP, S100B, and NSE**

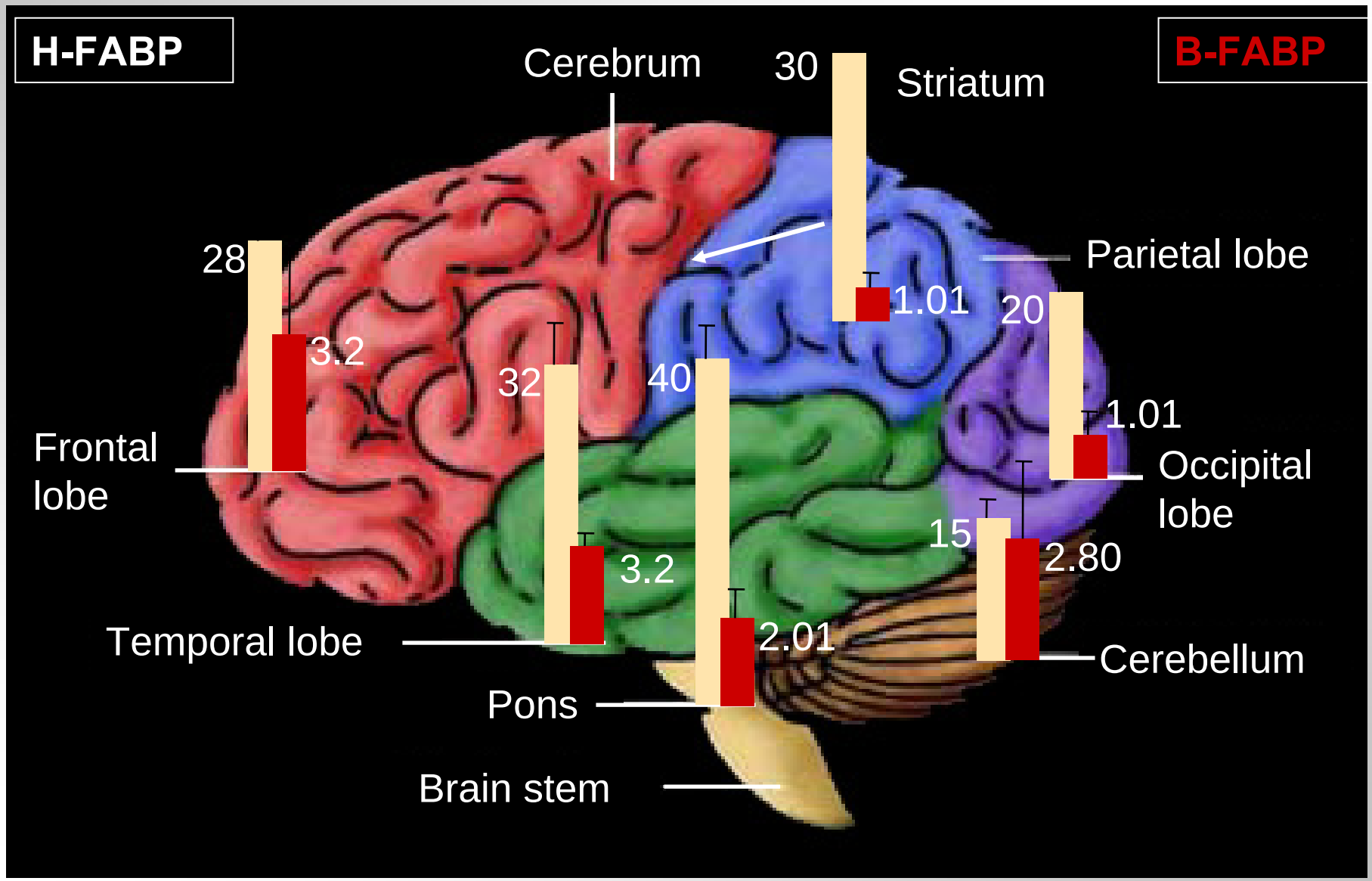
I.Tissue distribution:

- Tissue samples after autopsy or surgery (n=36, Bialystok, Poland)
- Frontal-, temporal-, occipital lobe, striatum, pons and cerebellum

B- and H-FABP tissue distribution (values in µg/g ww)



B- and H-FABP tissue distribution (values in µg/g ww)



II. Clinical evaluation:

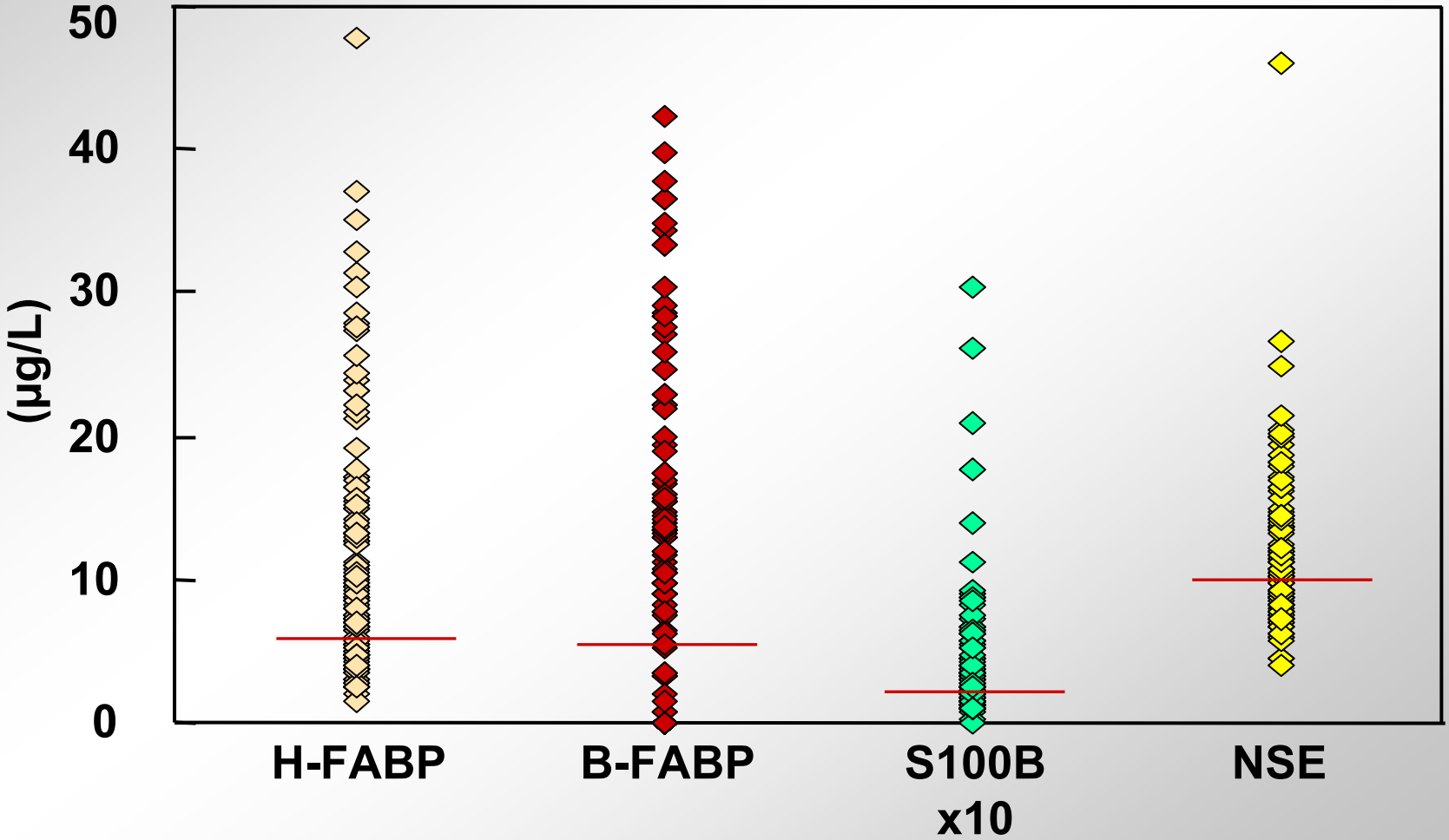
Mild traumatic brain injury

- Study group: serum samples from patients with Mild Traumatic Brain injury (MTBI)
- Inclusion criteria for patients (n=130)
  - 1) a blunt blow to the head resulting in post-traumatic amnesia of less than 1 h
  - 2) initial loss of consiousness of less than 15 min
  - 3) Glasgow Coma Scale score >13 on presentation
  - 4) absence of focal neurological signs

Serum samples were obtained within 6 h after trauma

Mild traumatic brain injury

Serum levels of different biochemical markers for brain injury in patients with mild traumatic brain injury



Mild traumatic brain injury

Percentage of patients with serum level above clinical cut-off value given for different biochemical marker proteins in Mild traumatic brain injury

Marker	Clinical cut off value	Patients above clinical cut off value
	(µg/L)	(%)
H-FABP	6	70 !
B-FABP	5	68 !
S100B	0.3	45
NSE	10	51

Electro-convulsive therapy (ECT)

- Study group Electro-convulsive therapy (ECT):
  - Electrical current ➔ brief seizure
  - Treatment of choice for depressive patients

- Inclusion criteria for patients (n=14)
  - Depressed patients undergoing bilateral ECT (on average 10 successive treatment sessions, 2 times a week) were studied
  - Serum samples (n=234) were obtained pre, 1 h and 3 h after ECT sessions (range 9-28 samples per patient)

Electro-convulsive therapy (ECT)

Percentage of patients with serum level above clinical cut-off value given for different biochemical marker proteins in ECT

Marker	Clinical cut off value	Patients above clinical cut off value	Range
	(µg/L)	(%)	(µg/L)
H-FABP	6	16.7 !	6.2-20.3
B-FABP	5	6.4 !	7.2-50.5
S100B	0.3	0.4	0-0.35

Study group:

- Cerebro-vascular accident (CVA):
  - Blood supply disturbed ➔ cell death/damage

- Inclusion criteria for patients:
  - Serum was obtained from 12 CVA patients, 3 patients were treated with thrombolyticum within 3 h after onset of symptoms
  - Samples taken at 6, 24, 48 and 72 h after treatment

Cerebro-vascular accidents (CVA)

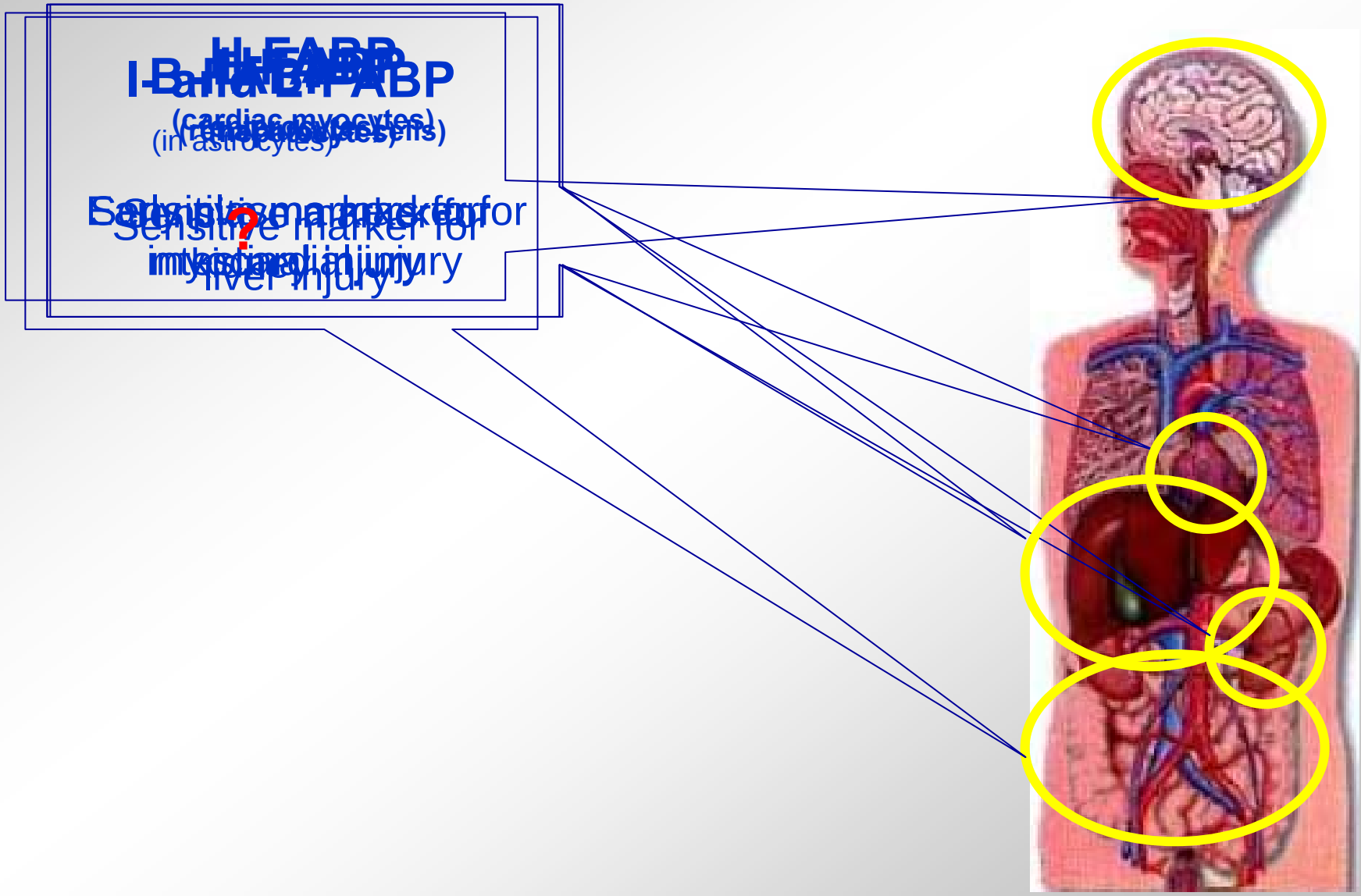
Percentage of patients with serum level above clinical cut-off value given for different biochemical marker proteins in CVA

Marker	Clinical cut off value	Patients above clinical cut off value	Range
	(µg/L)	(%)	(µg/L)
H-FABP	6	67 !	1.72-24.3
B-FABP	5	56 !	0-155
S100B	0.3	22	0.05-0.47
NSE	10	0	6.0-9.3

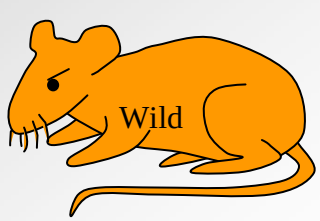
Conclusions

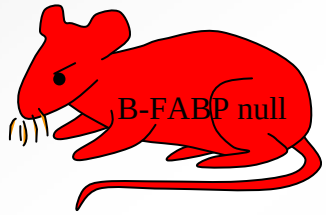
- H-FABP and B-FABP are more sensitive markers of brain injury than S100B or NSE
- H-FABP release in ECT is not from skeletal muscle damage

FABP release due to organ injury



Normal brain architectures in B-FABP null mice

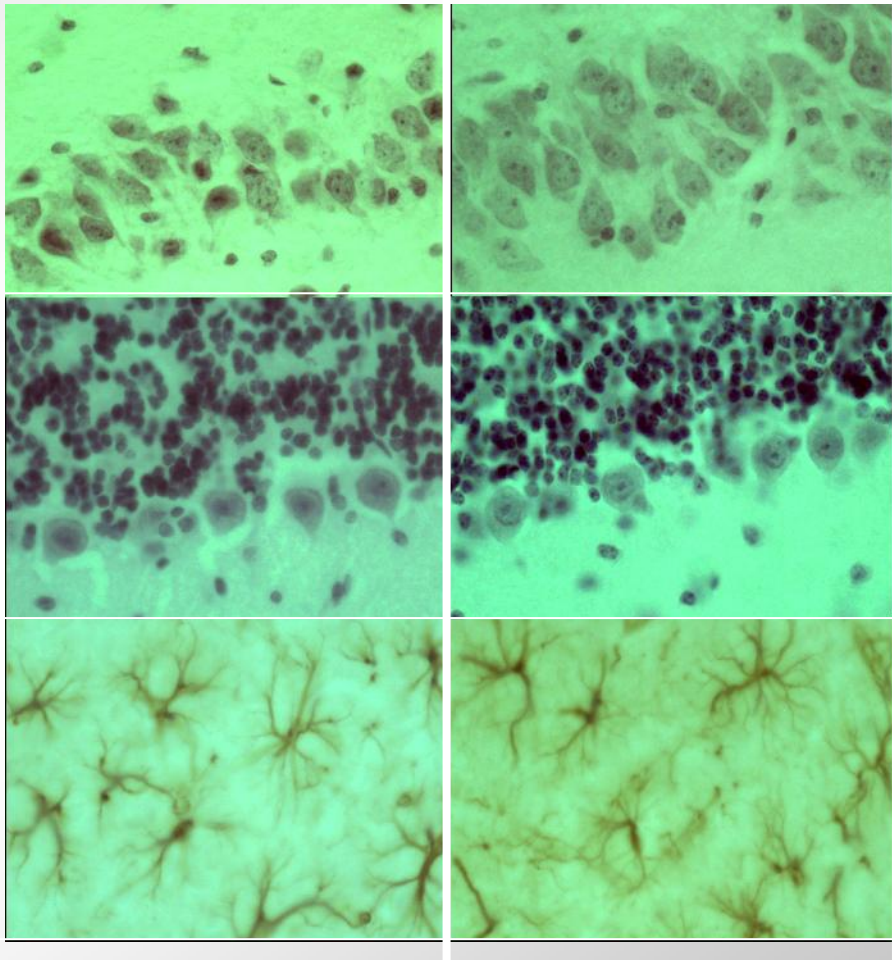




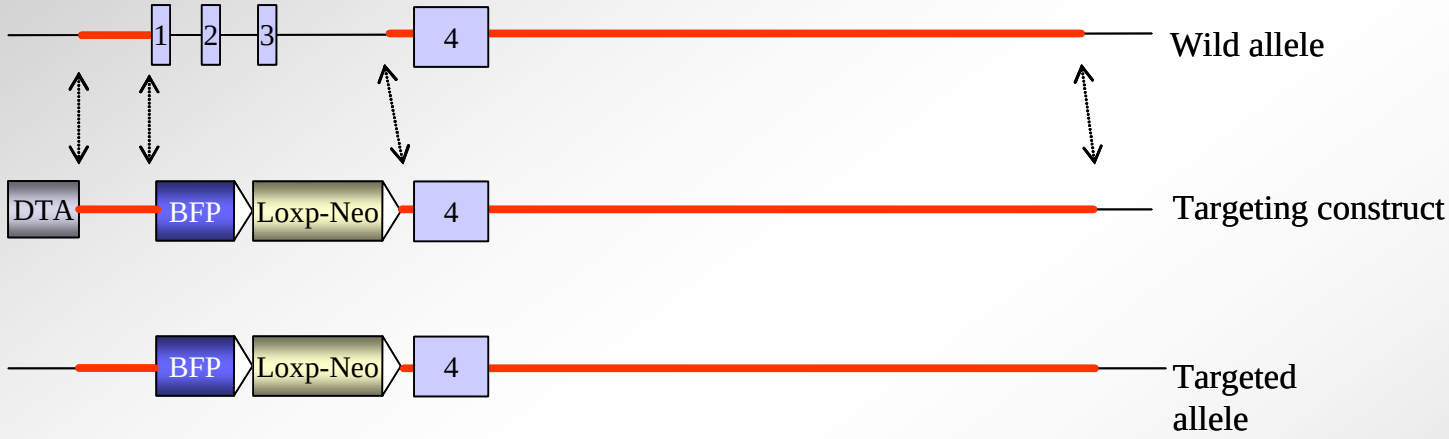
hippocampus

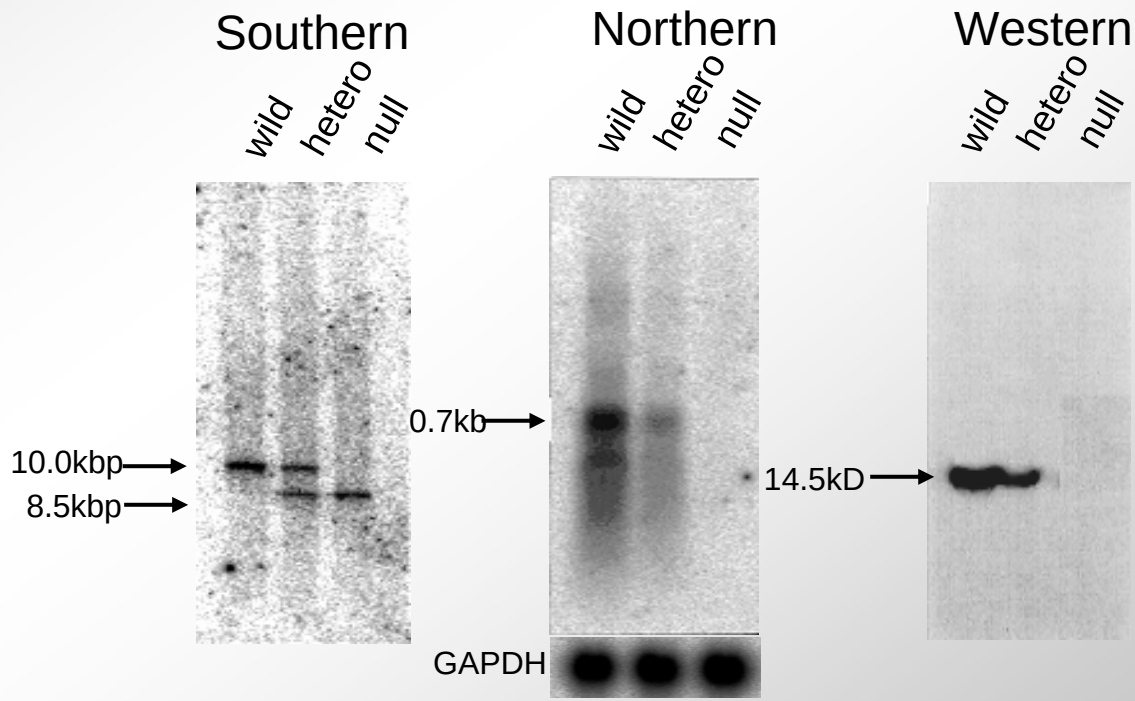
cerebellum (H&E)

cerebral cortex (glial cells)



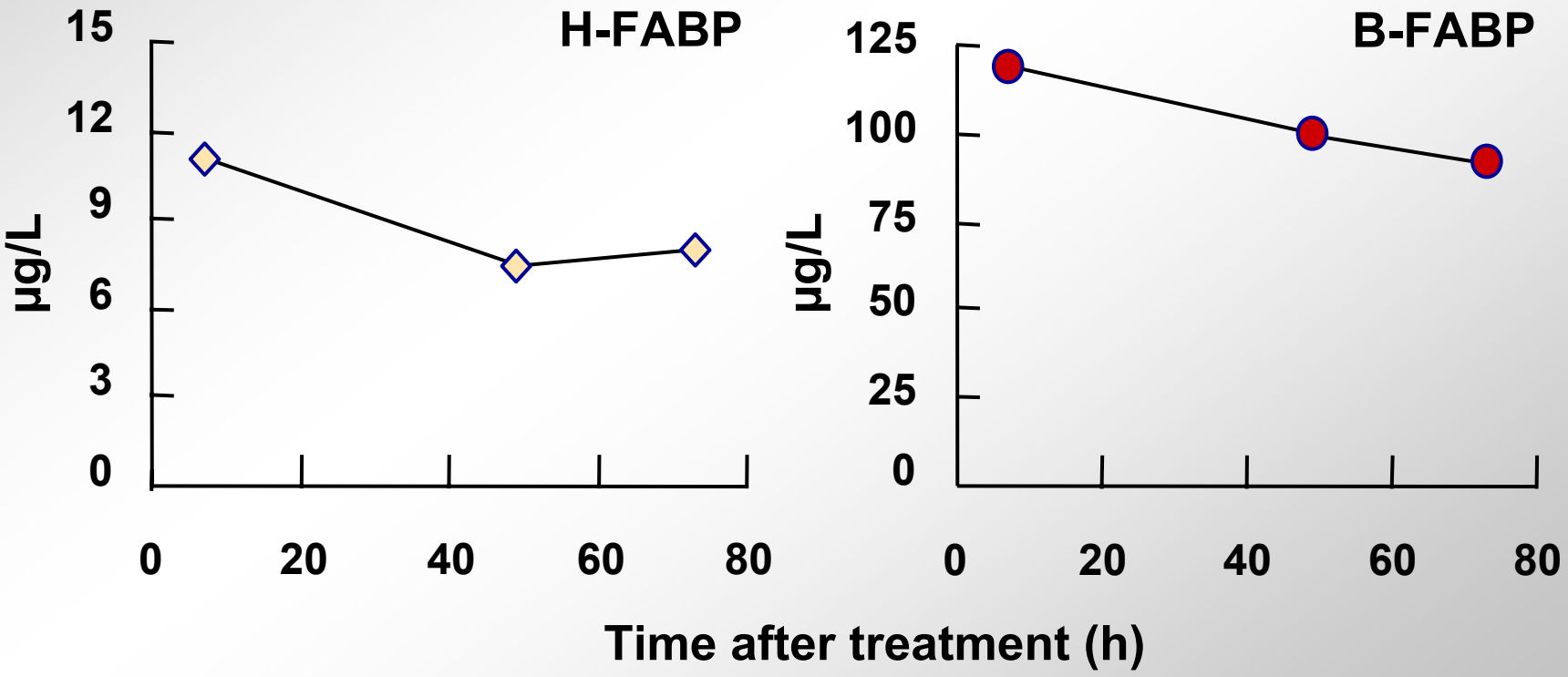
Targeting strategy of mouse B-FABP gene





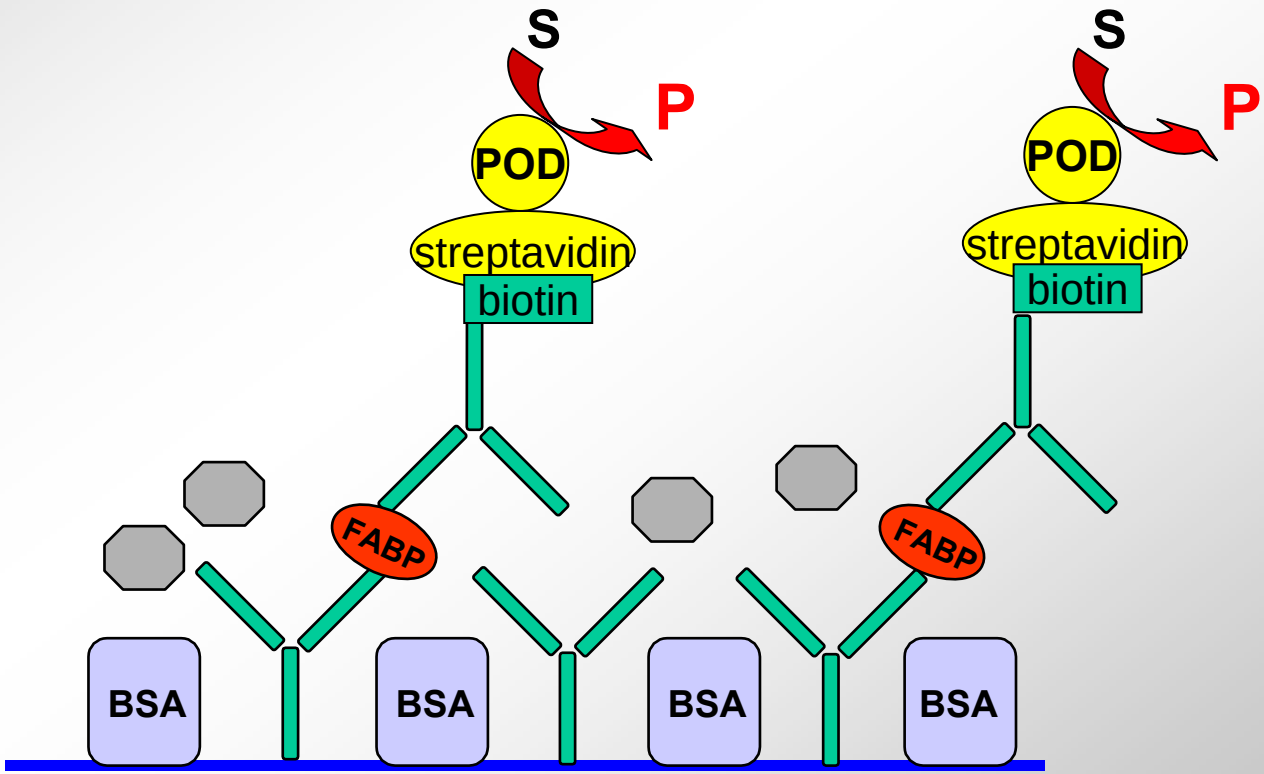
Cerebro-vascular accidents (CVA)

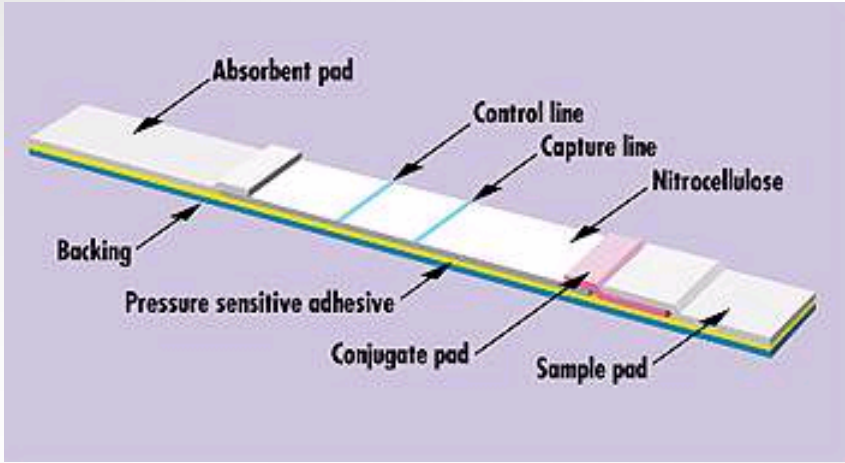
Release curve of H- and B-FABP, in serum of a typical CVA patient



Sandwich ELISA

Antigen capture antibody enzyme-linked immunosorbent assay





www.rennesens.de