Nanopartikel als Träger zum Transport von Arzneistoffen über die Blut/Hirn-Schranke

materials valley

Hanau/Wolfgang 21. Februar 2013

JÖRG KREUTER

INSTITUT FÜR PHARMAZEUTISCHE TECHNOLOGIE

JOHANN WOLFGANG GOETHE-UNIVERSITÄT FRANKFURT

DRUG TRANSPORT ACROSS THE BLOOD-BRAIN BARRIER WITH NANOPARTICLES

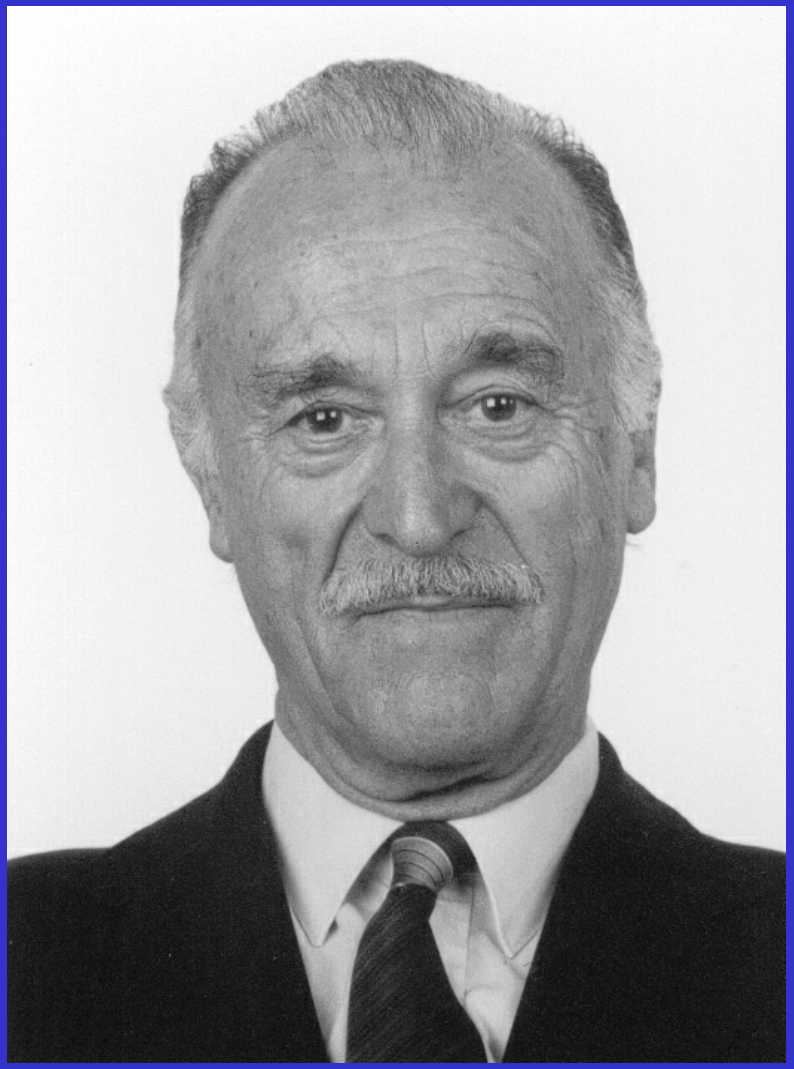
- Definition of nanoparticles
- Brain delivery with nanoparticles
- Chemotherapy
- Toxicology
- Mechanism
- Attachment of targeting ligands

DEFINITION OF NANOPARTICLES

Nanoparticles are solid polymeric particles of a size between 10 and 1000 nm into which drugs or biologically active materials are incorporated, surface adsorbed or chemically bound.

NANO-PARTICLES

PETER SPEISER



DRUG TARGETING

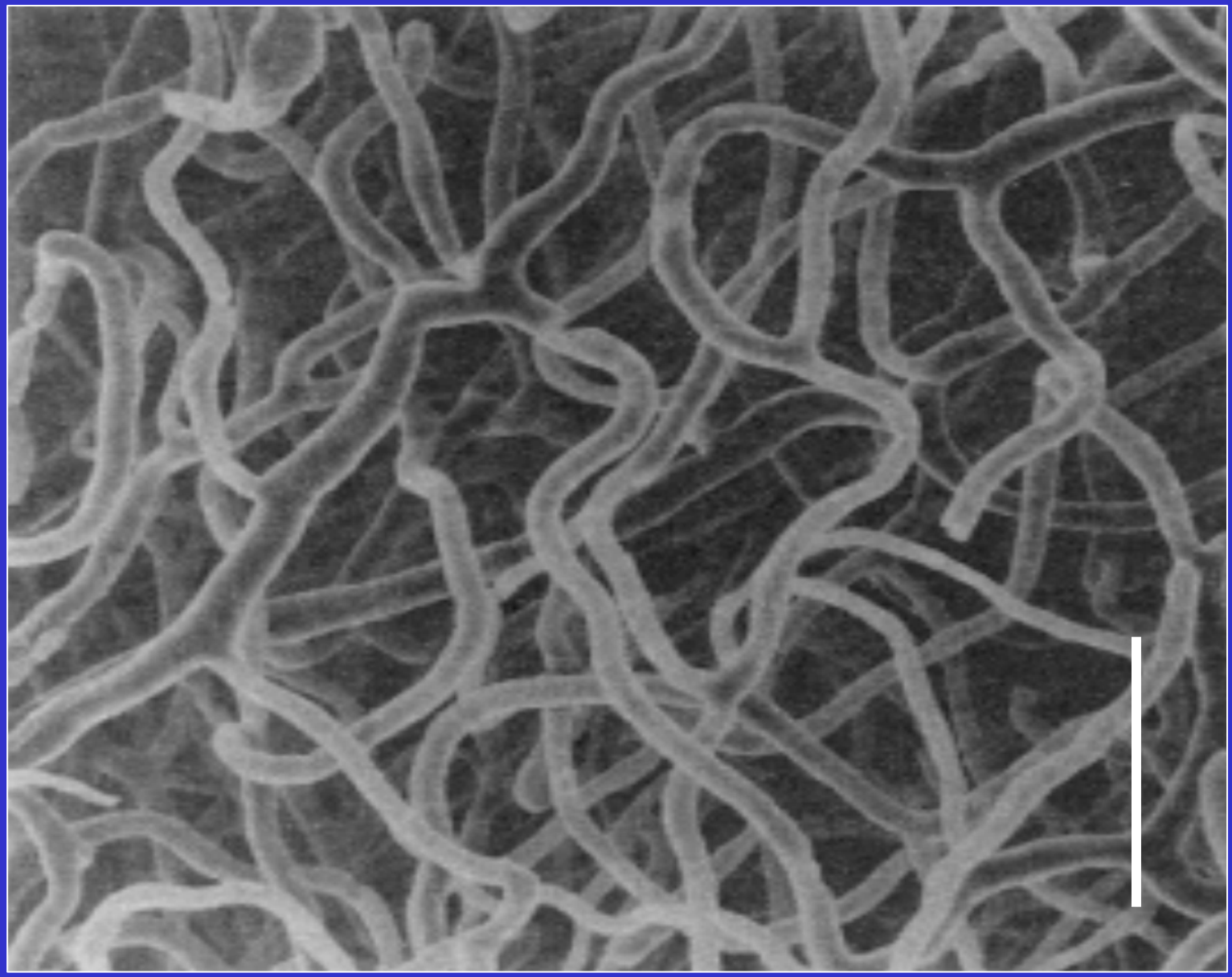
PAUL EHRLICH

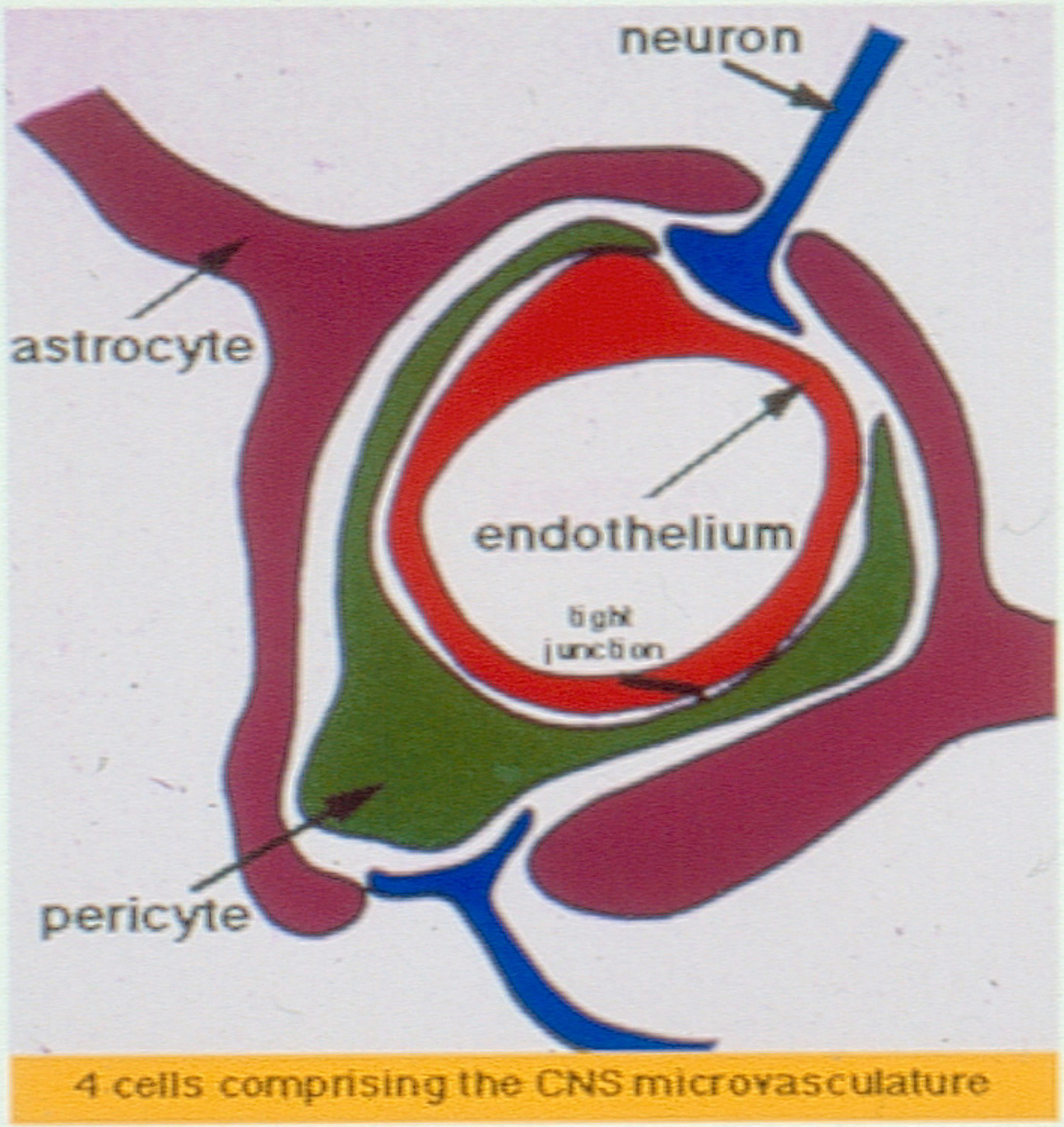
STRUCTURE OF THE BLOOD-BRAIN-BARRIER

Scanning Electron Micrograph

Cast of Rat Thalamus

Bar =50μm



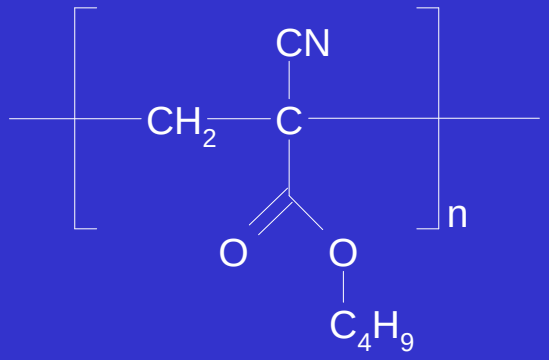


4 cells comprising the CNS microvasculature

BRAIN DELIVERY WITH NANOPARTICLES

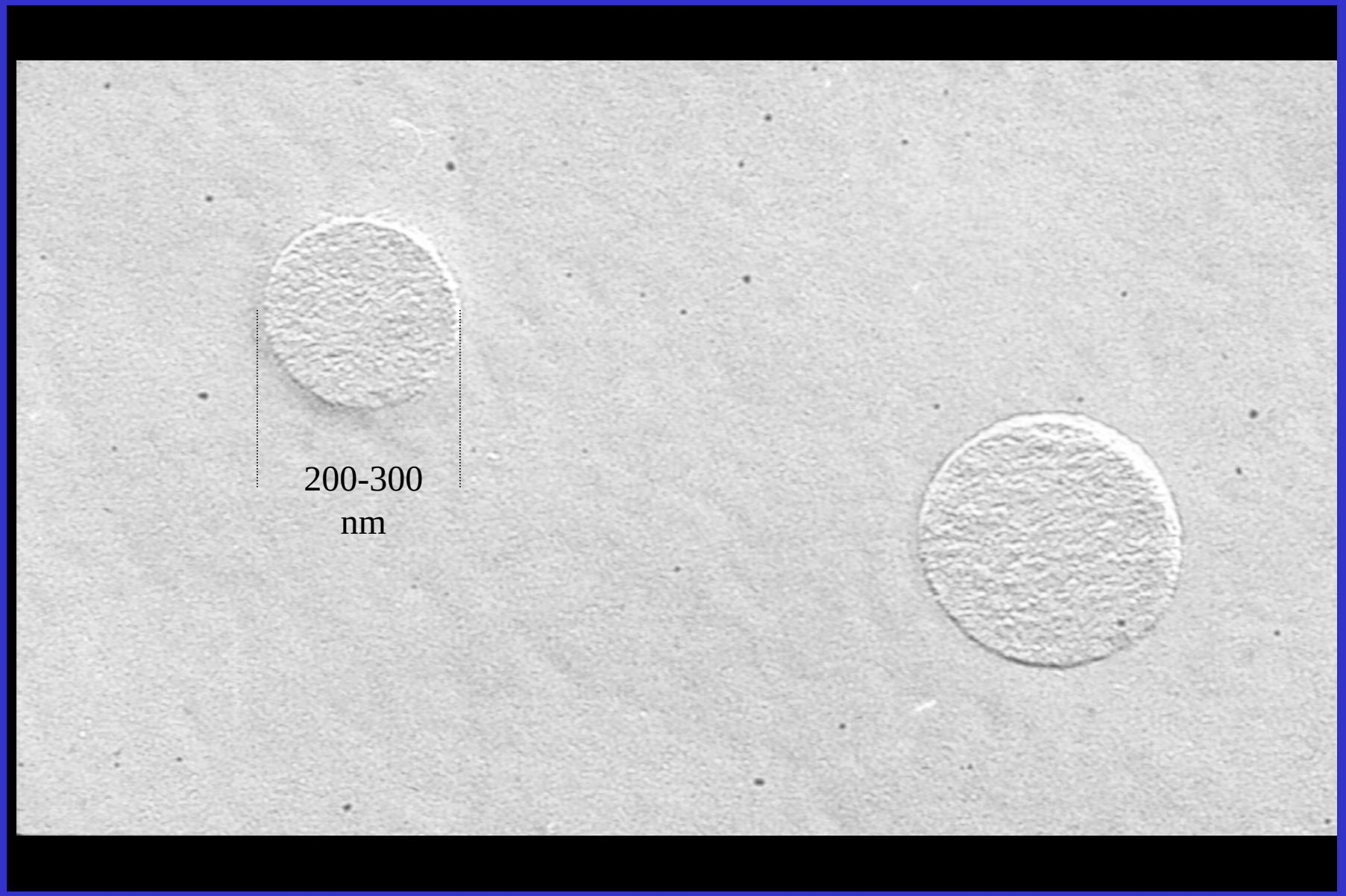
Type of Nanoparticles Used

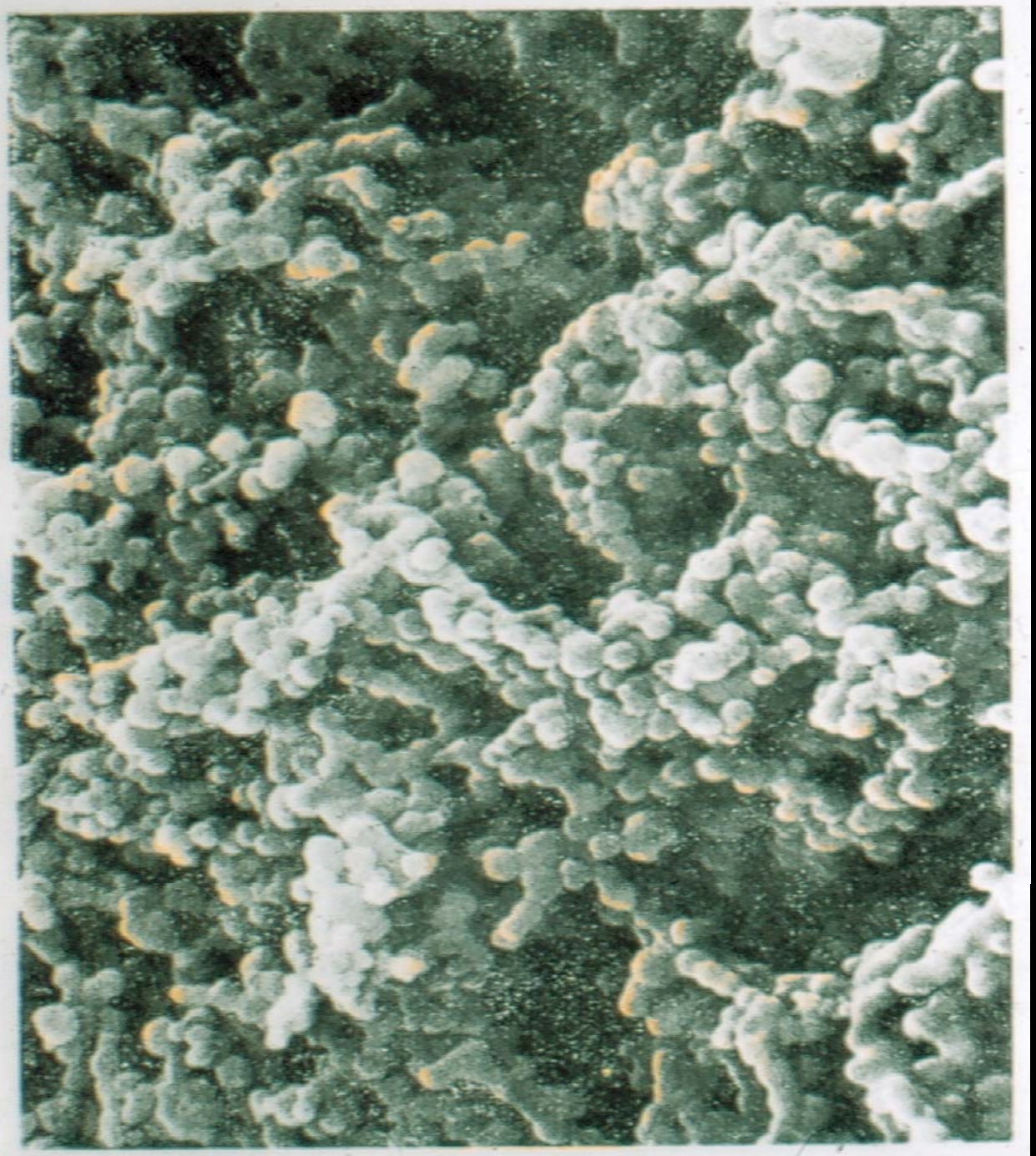
- **Poly(butyl cyanoacrylate)**

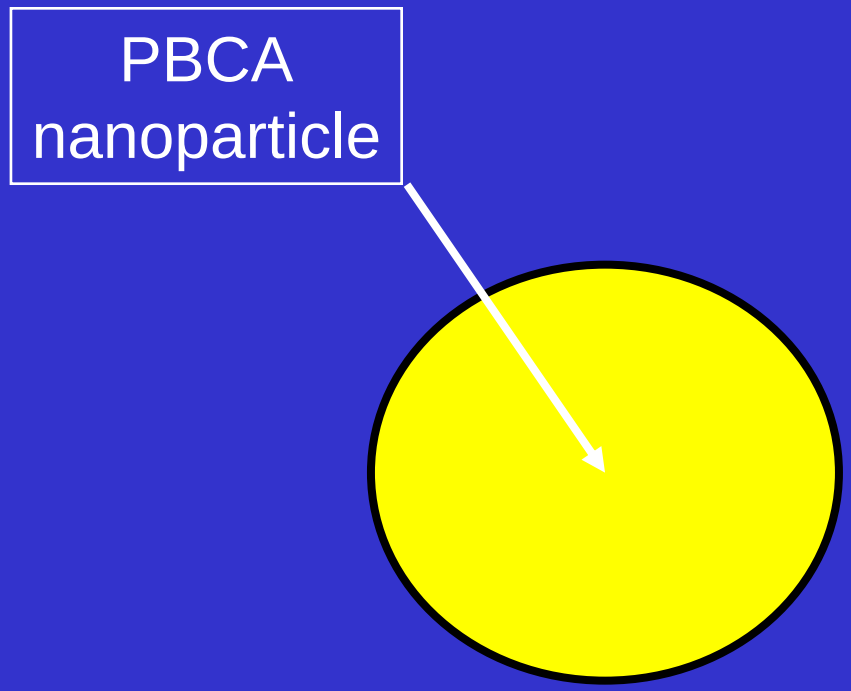


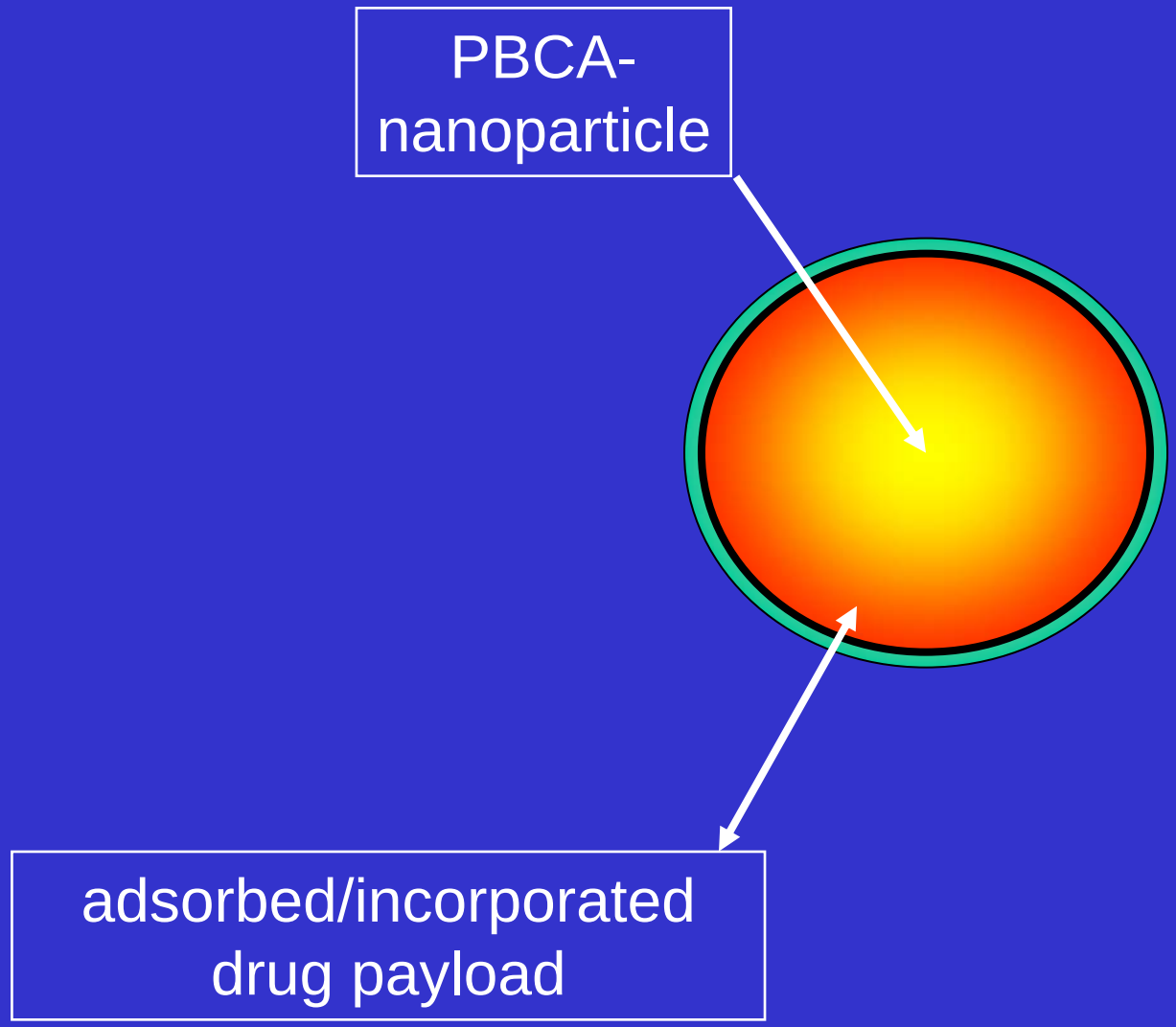
- **Particle size : 230 – 250 nm**
- **Monolithic particles**

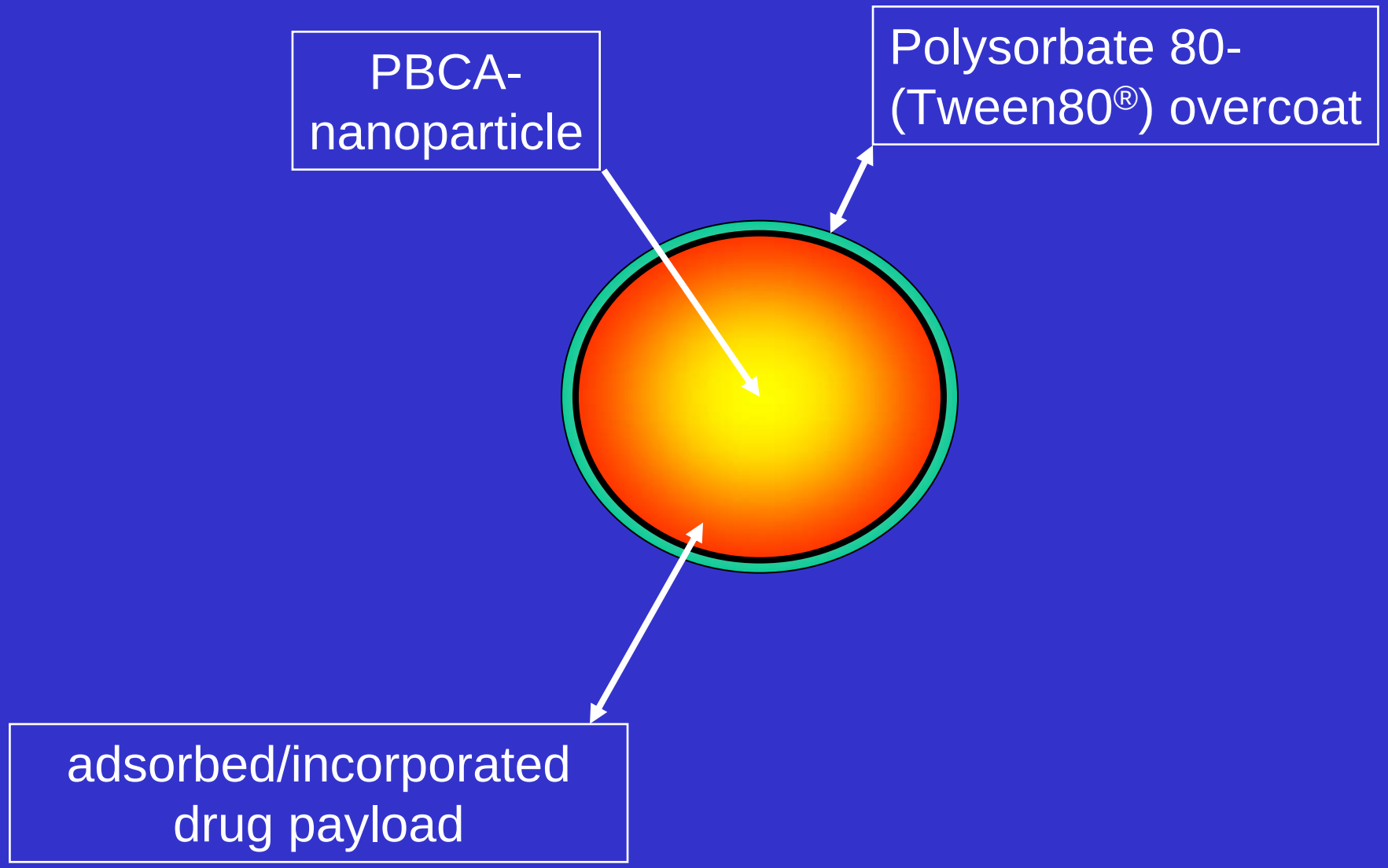
EM Freeze Fracture of Nanoparticles



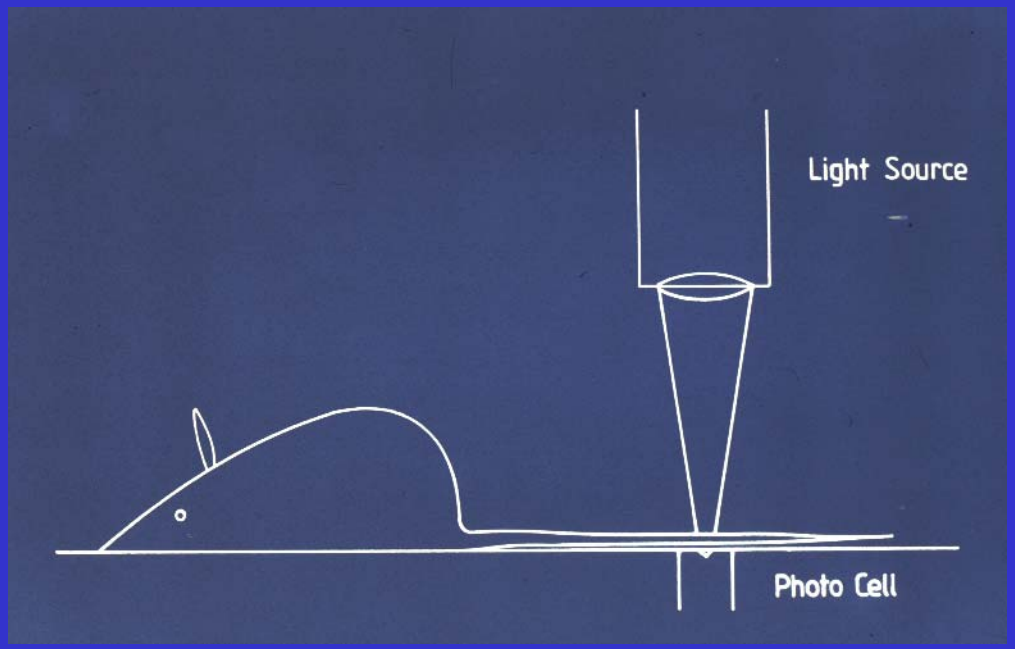



PBCA
nanoparticle


PBCA-
nanoparticle
adsorbed/incorporated
drug payload


PBCA-nanoparticle
Polysorbate 80-(Tween80®) overcoat
adsorbed/incorporated drug payload

Mouse Tail-Flick Analgesia Test



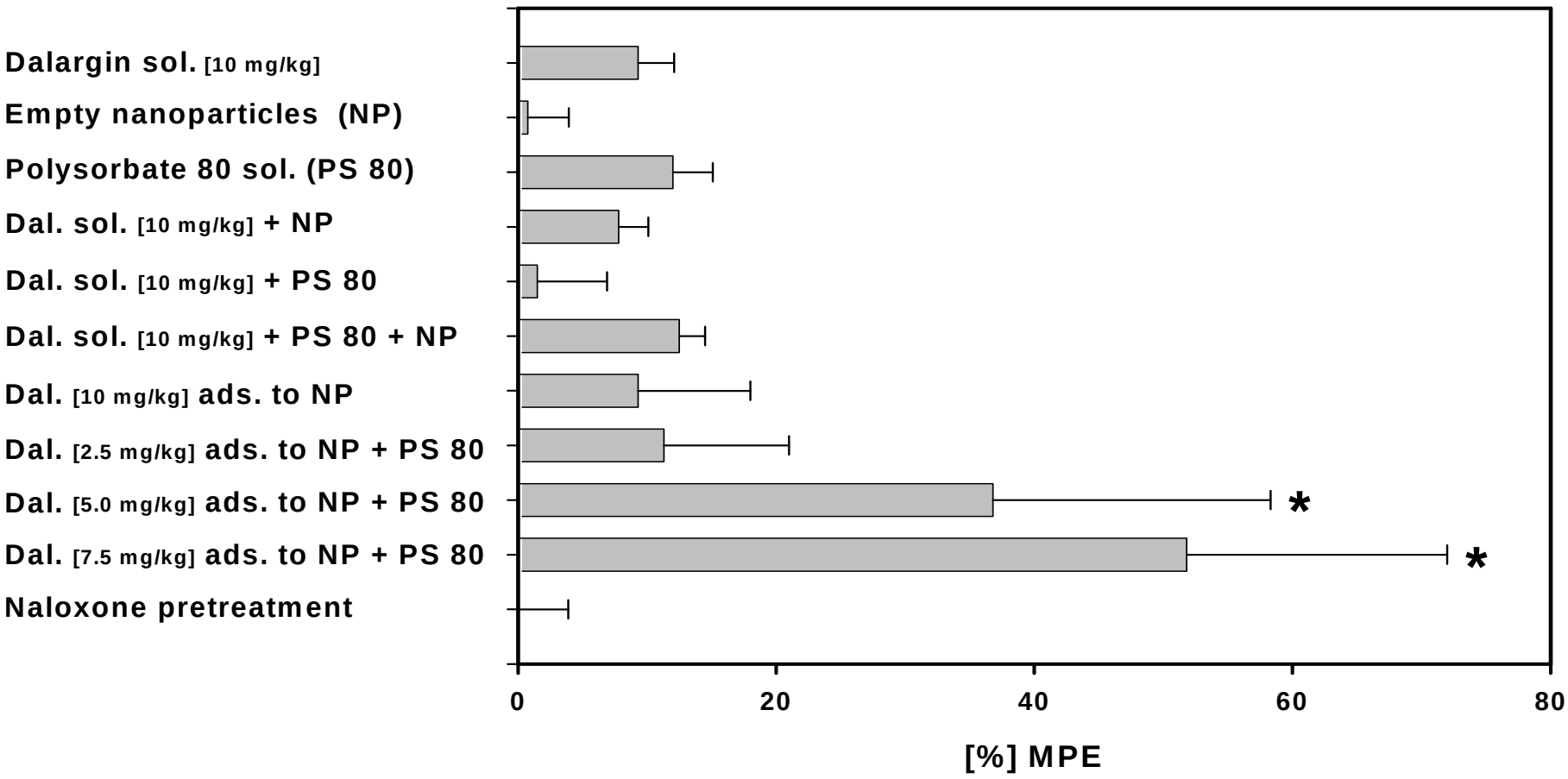
Maximally Possible Effect

ANALGESIC (ANTINOCICPETIVE) EFFECTS IN MICE – TAIL FLICK TEST

Maximally Possible Effect

$$\%\ \text{MPE} = \frac{\text{POST DRUG LATENCY} - \text{PREDRUG LATENCY}}{\text{CUTOFF TIME} - \text{PREDRUG LATENCY}} \cdot 100$$

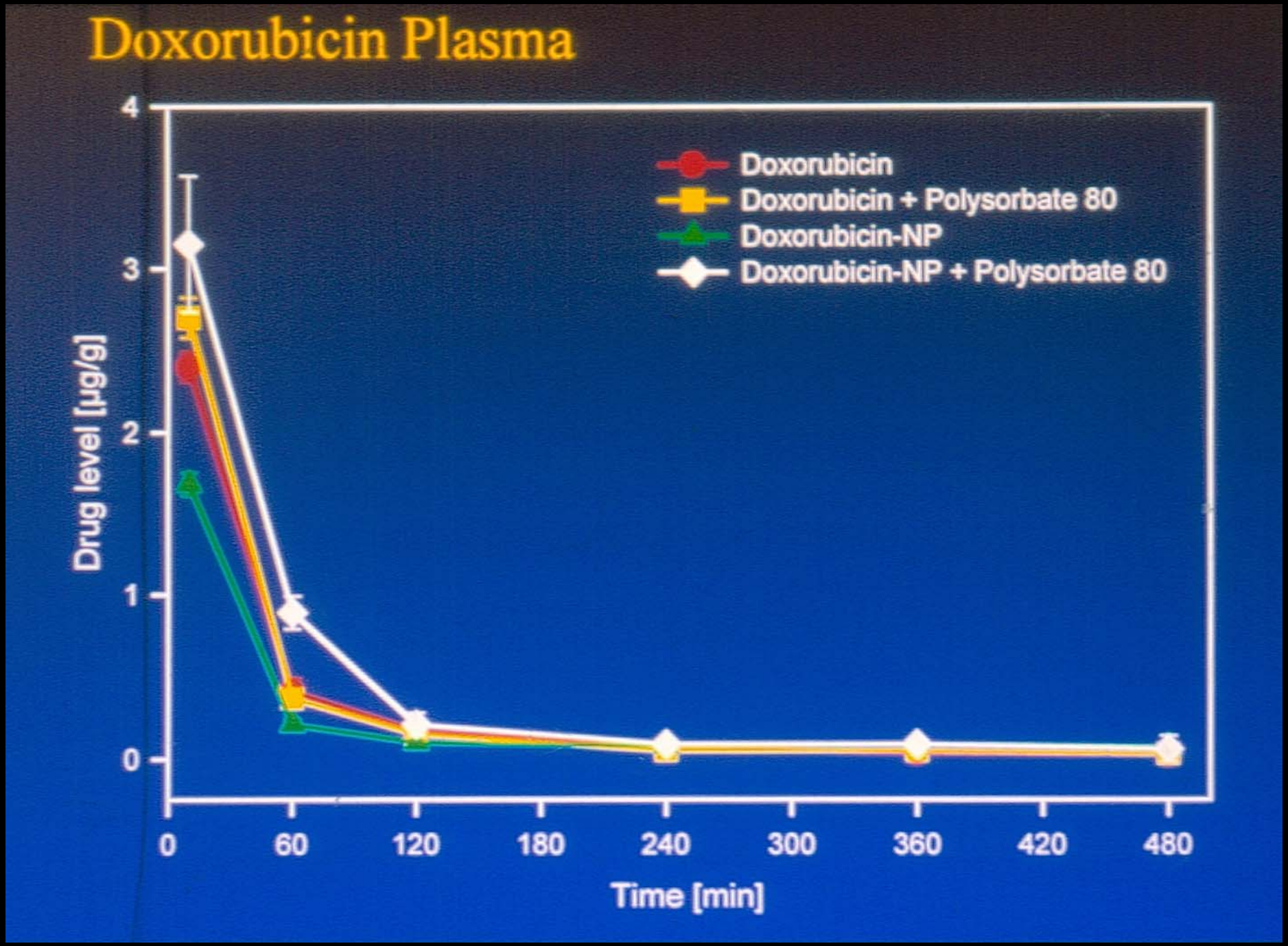
ANALGESIC (ANTINOCICPETIVE) EFFECTS IN MICE – TAIL FLICK TEST



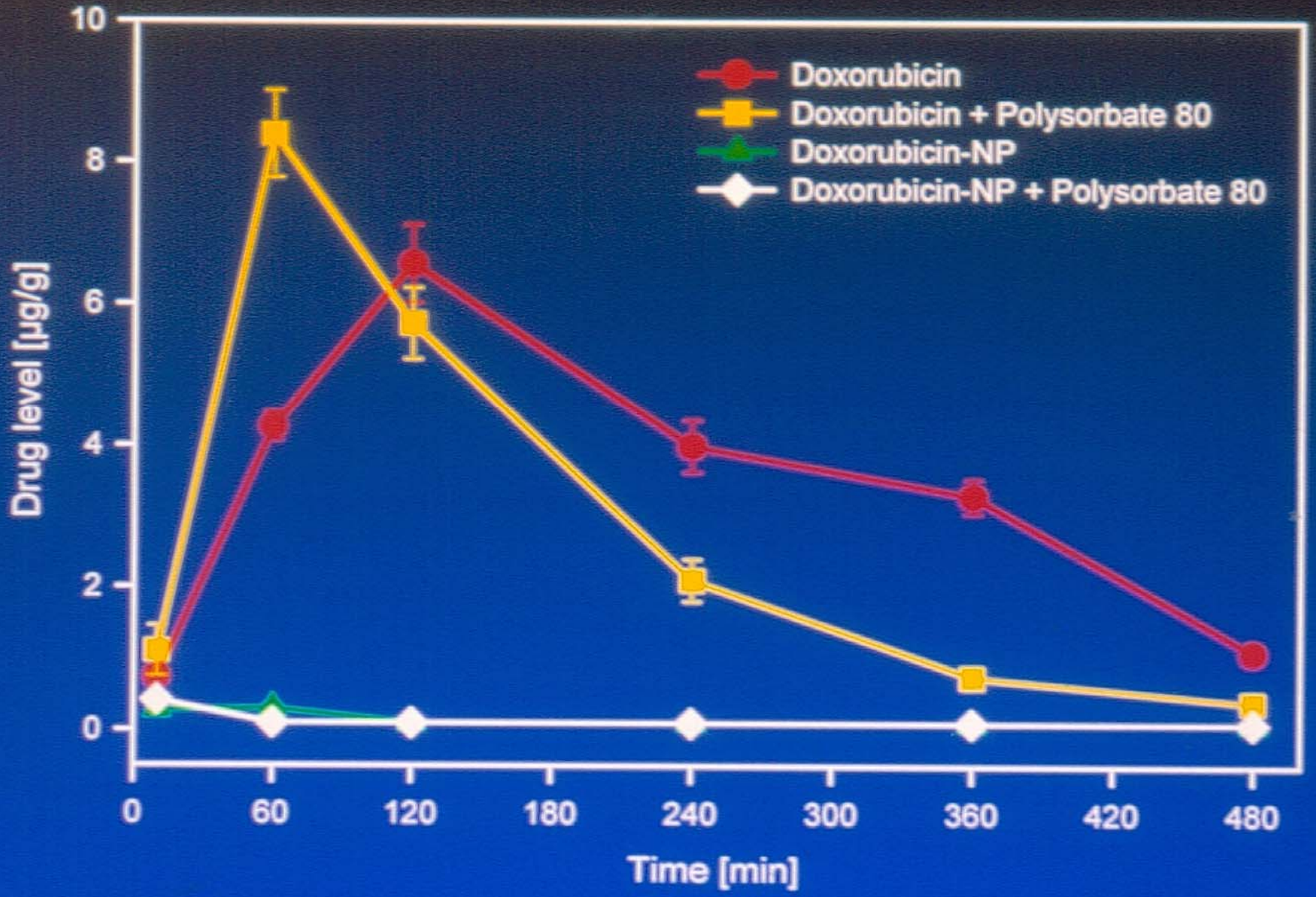
DRUGS DELIVERED ACROSS THE BLOOD-BRAIN BARRIER WITH NANOPARTICLES

- KYOTORPHIN (dipeptide)
- LOPERAMIDE
- TUBOCURARINE
- MRZ 2/576 AND MRZ 2/596 (NMDA-receptor antagonists)
- DOXORUBICIN

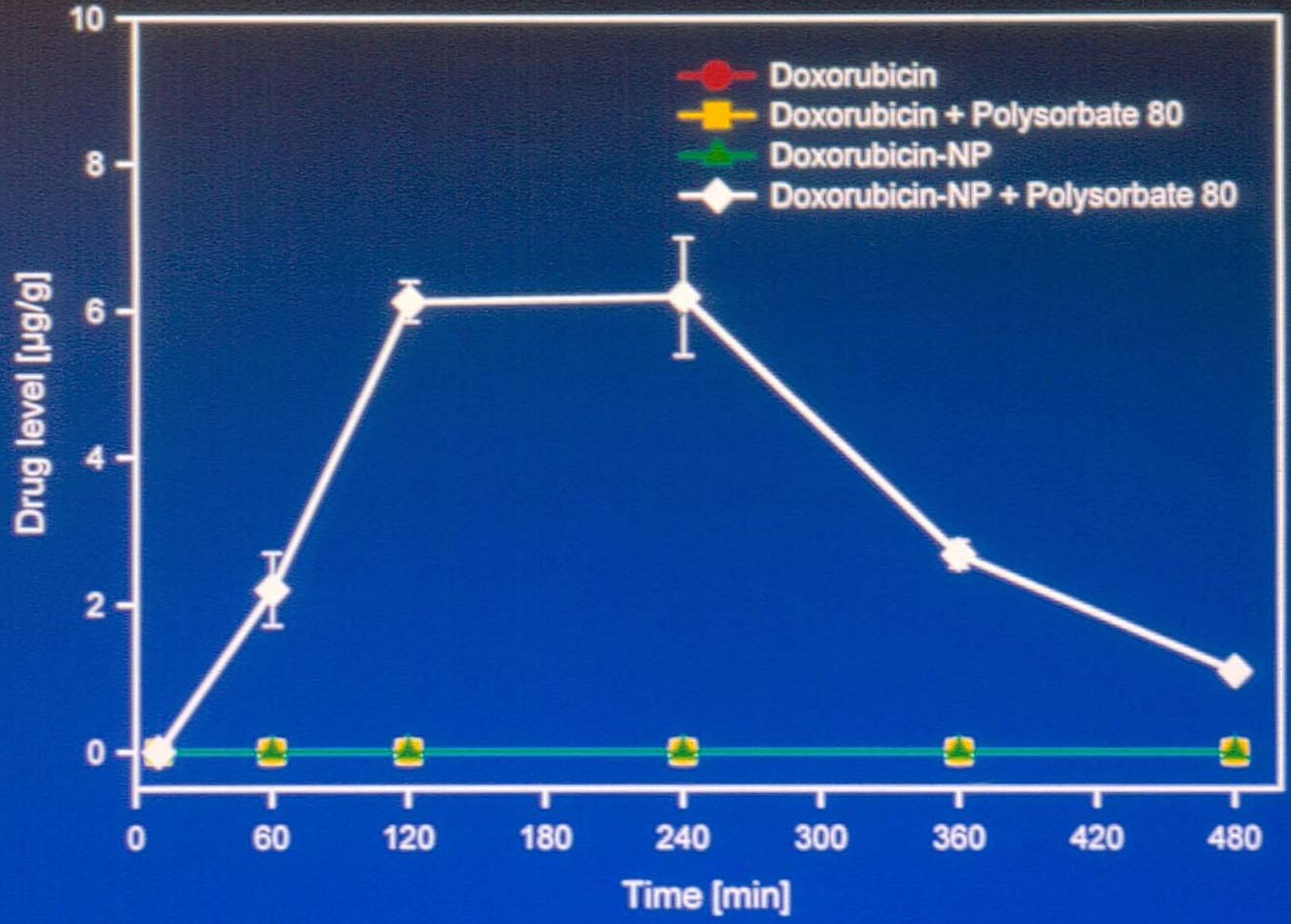
Doxorubicin Plasma



Doxorubicin Heart

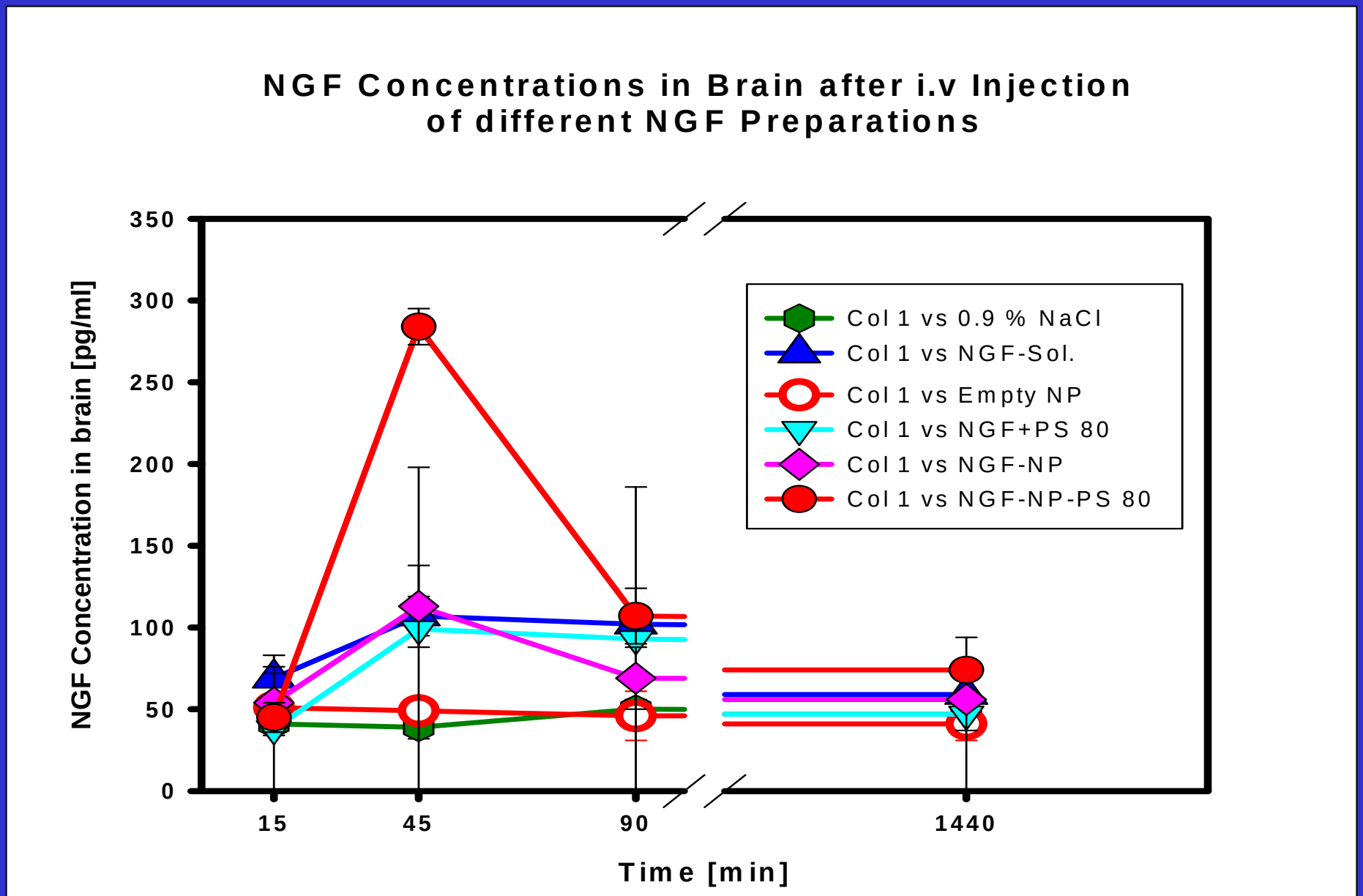


Doxorubicin Brain

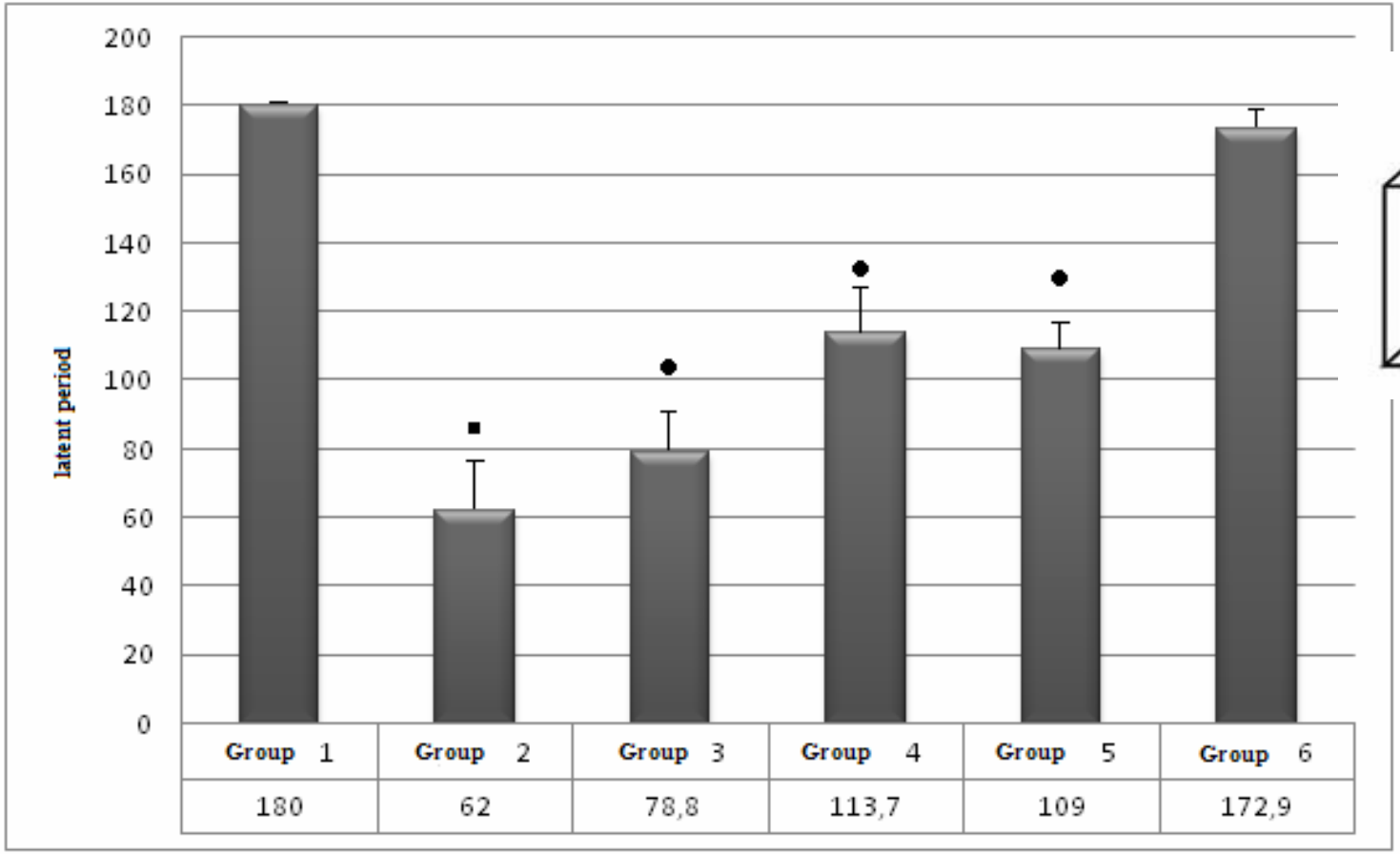


LARGE PEPTIDES

NGF, MW ~ 130 kDa
rhoGin MW ~ 21 kDa


NGF Concentrations in Brain after i.v Injection of different NGF Preparations
NGF Concentration in brain [pg/ml]
350
300
250
200
150
100
50
0
15
45
90
1440
Time [min]
Col 1 vs 0.9 % NaCl
Col 1 vs NGF-Sol.
Col 1 vs Empty NP
Col 1 vs NGF+PS 80
Col 1 vs NGF-NP
Col 1 vs NGF-NP-PS 80

Latent Period in the Passive Avoidance Test [sec]



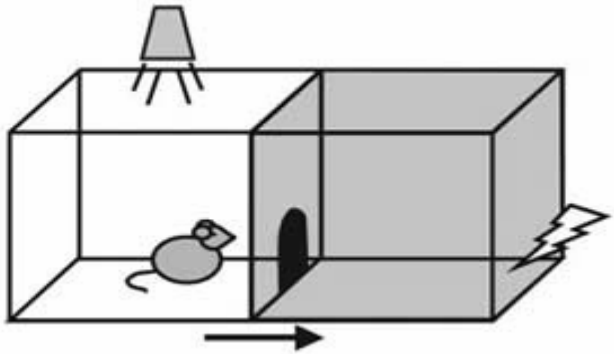
Group 1	Group 2	Group 3	Group 4	Group 5	Group 6
180	62	78,8	113,7	109	172,9

Mean latent period (seconds ± m)

Group 1 passive control Group 2 control of amnesia Group 3 NGF (5 µg/mice, i.v.).
Group 4 NGF (5 µg/mice mixed with 1 % polysorbate-80 solution, i.v.).
Group 5 NGF (5 µg/mice, adsorbed on nanoparticles, i.v.)
Group 6 NGF (5 µg/mice, adsorbed on nanoparticles coated with polysorbate-80, i.v.).

● Statistically reliable difference from group 6 $p \leqslant 0.05$

■ Statistically reliable difference from group 6 $p \leqslant 0.01$

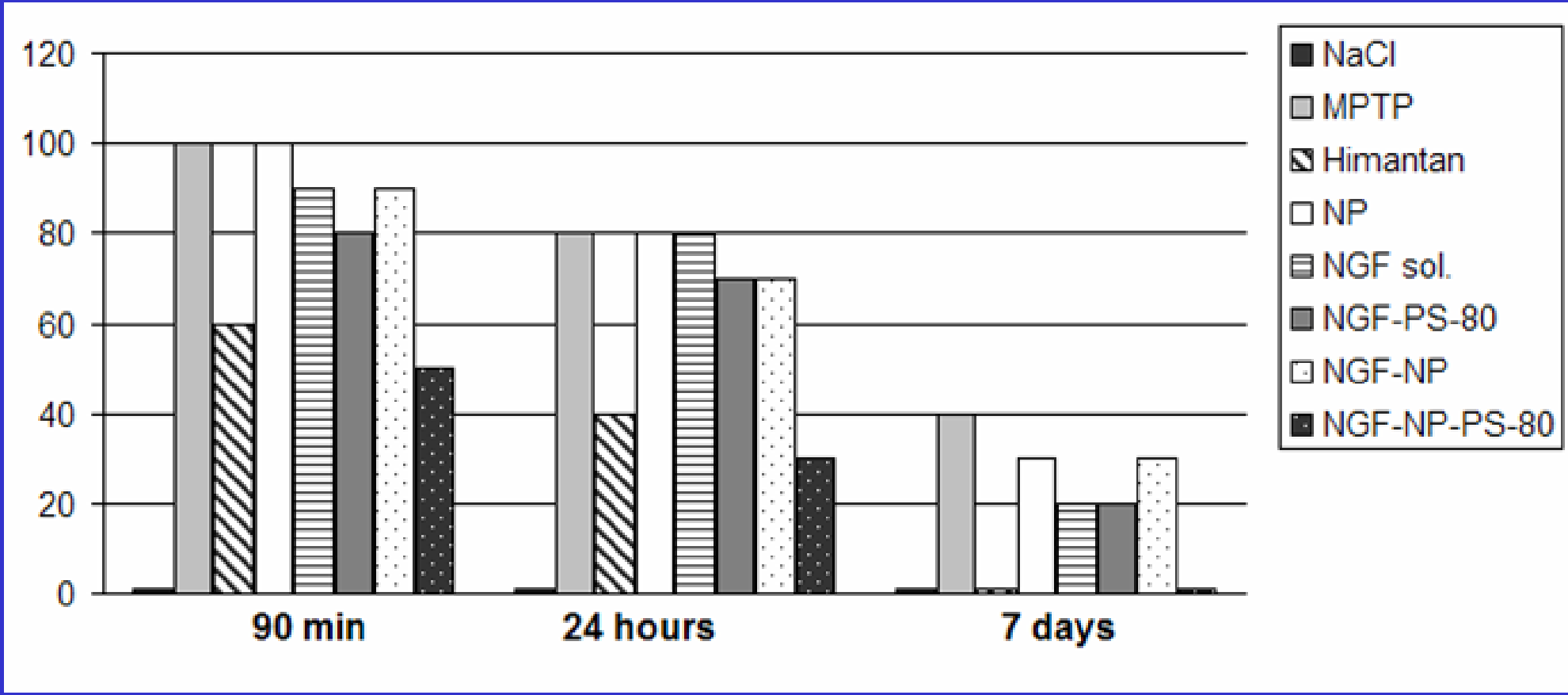


Passive Avoidance

Exploits a natural tendency of mice to enter dark environments.

Unidirectional: mouse goes from light to dark chamber.

Weighted tremor score [%]



CHEMOTHERAPY

CHEMOTHERAPY

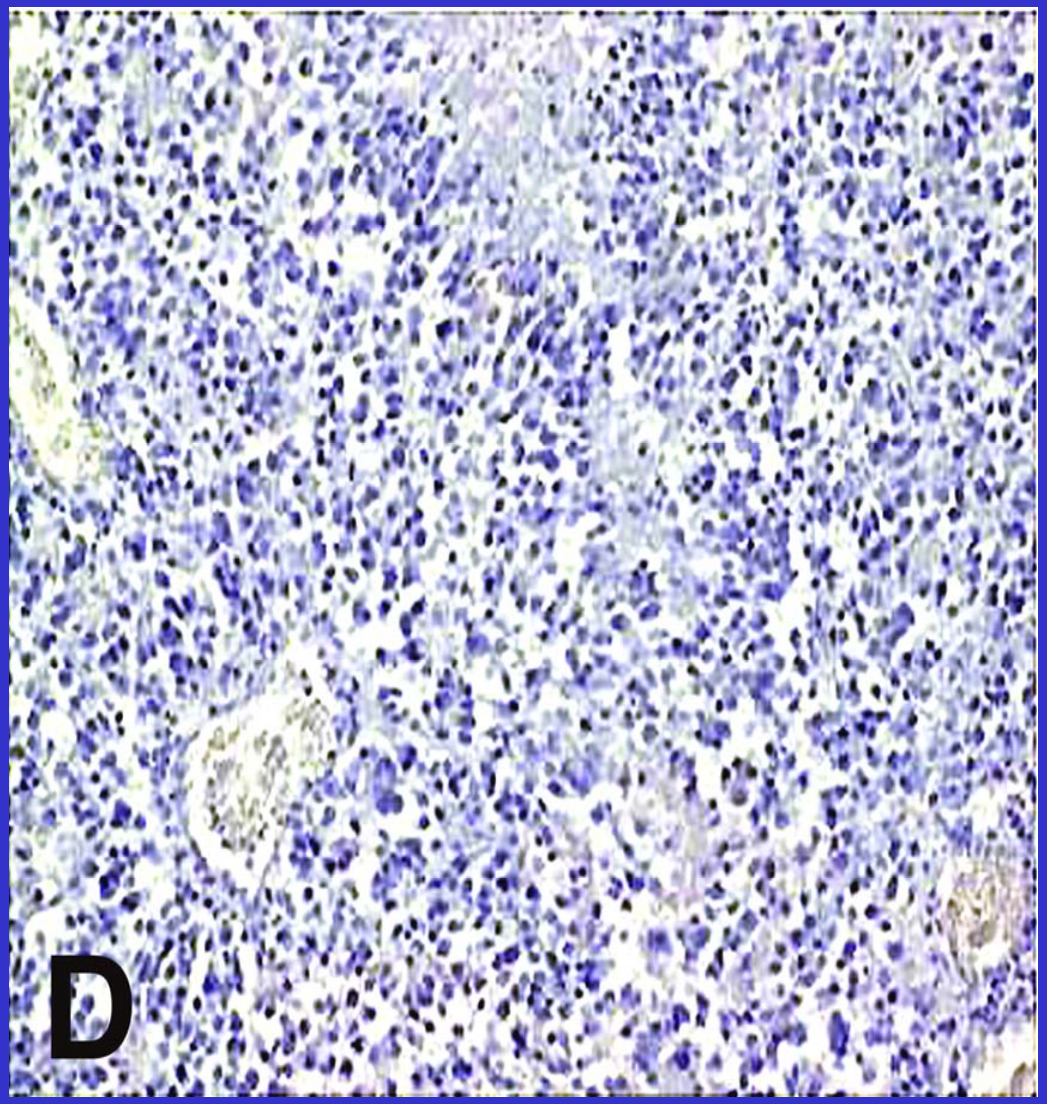
- **Preparations:**

  1) Untreated control,
  2) Blank NP coated with polysorbate 80 (NP+PS),
  3) Doxorubicin in saline (DOX),
  4) Doxorubicin in 1% polysorbate 80 solution (DOX+PS)
  5) Doxorubicin bound to NP (DOX-NP),
  6) Doxorubicin bound to NP coated with polysorbate 80 (DOX-NP+PS).

CHEMOTHERAPY

- 101/8 glioblastoma-bearing animals
- 6 groups (n = 10 - 26)
- Intravenous injection into the tail vein
- 1.5 mg/kg doxorubicin
- Day 2, day 5, and day 8.

Histological evaluation of brain from rats with 101/8 glioblastoma

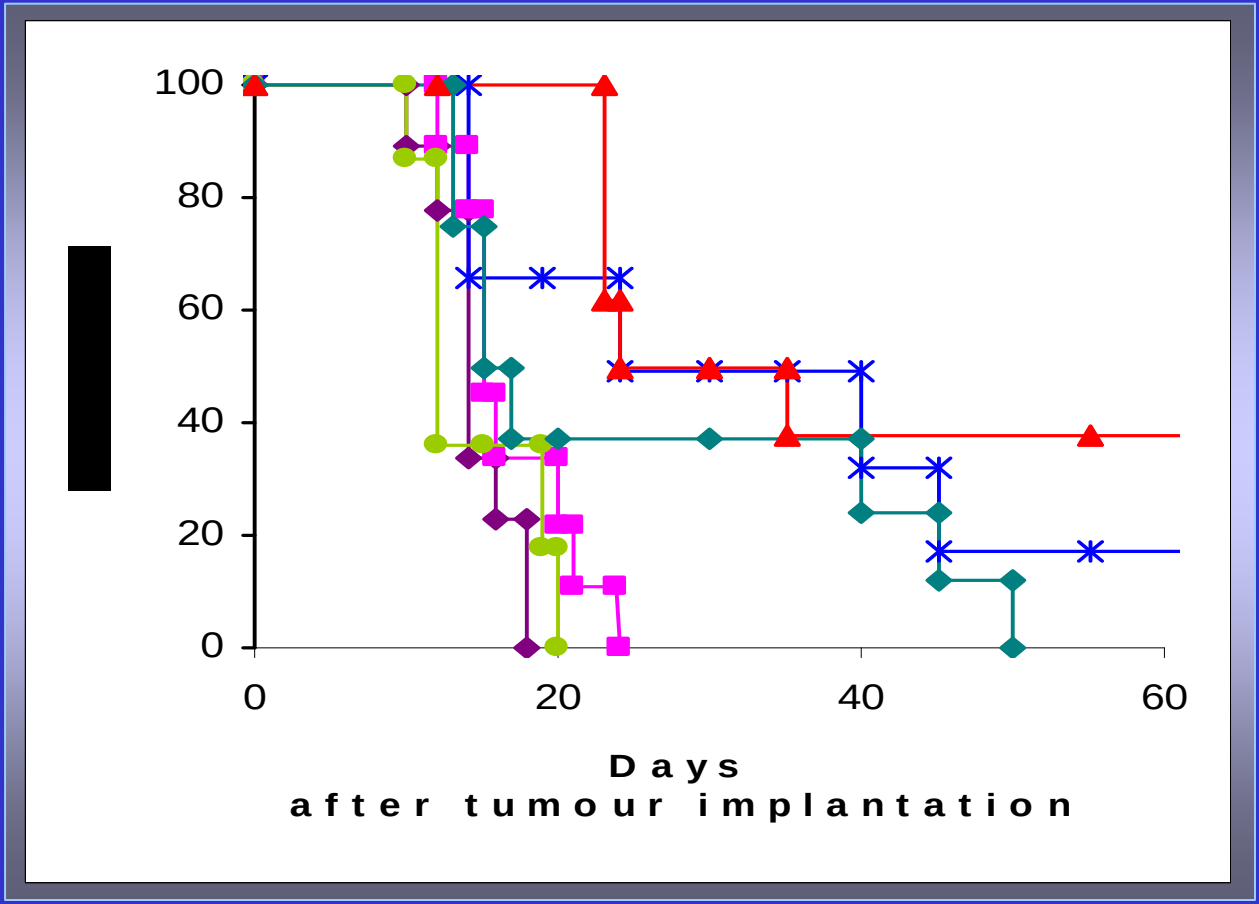


Untreated control: a pattern similar to human malignant gliomas, with diffuse growth and significant amounts of necrosis

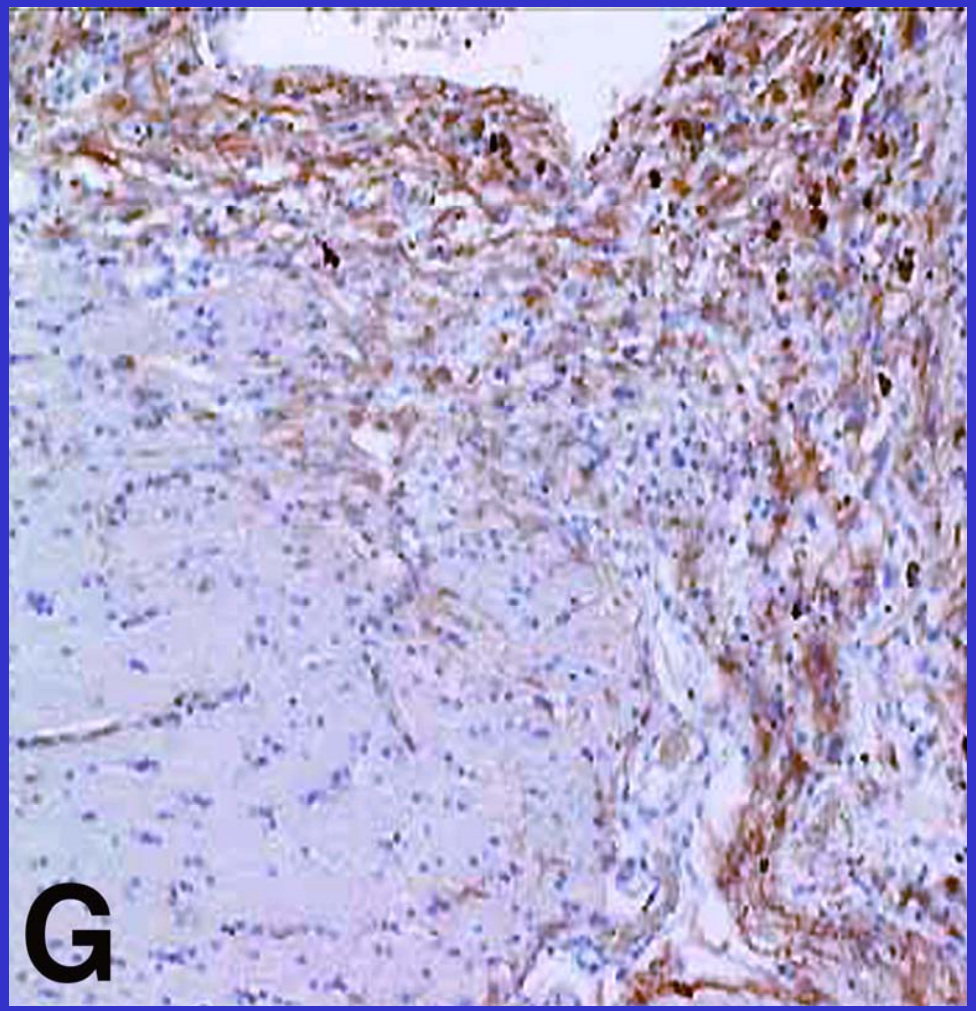
EFFICACY OF DOX PREPARATIONS IN RATS BEARING INTRACRANIAL 101/8 GLIOBLASTOMA:

CONTROL: ◆ NP+Ps: ● DOX+Ps: X

DOX-NP: ◆ DOX: ■ DOX-NP+Ps: ▲

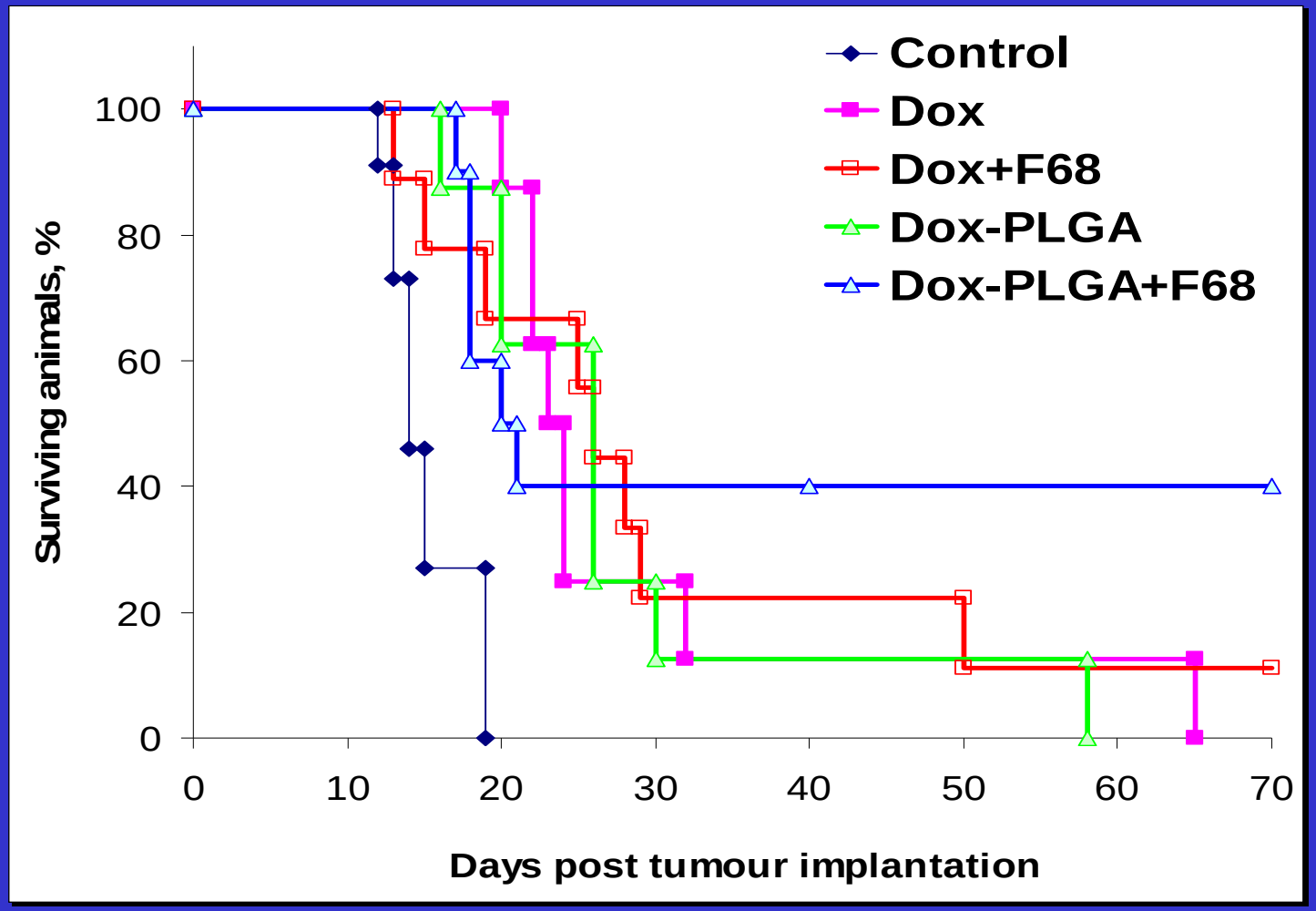


Histological evaluation of brain from long-surviving rats with 101/8 glioblastoma



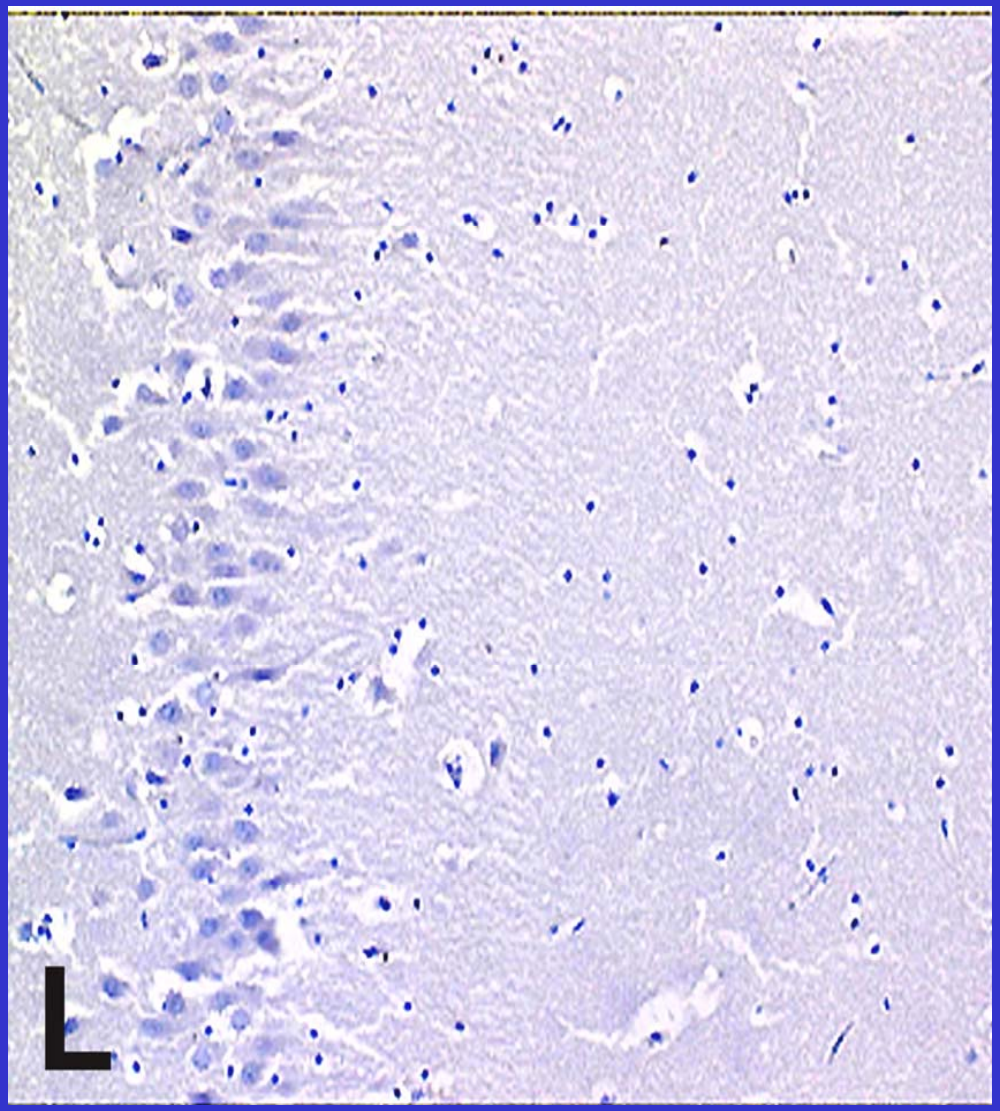
Absence of tumour
Gliotic cerebral scar at the cite of implantation

Chemotherapy of intracranial 101/8 glioblastoma in rats using doxorubicin loaded into PLGA nanoparticles: Effect of Pluronic® F68 coating



TOXICITY

Histological evaluation of brain from rats with 101/8 glioblastoma

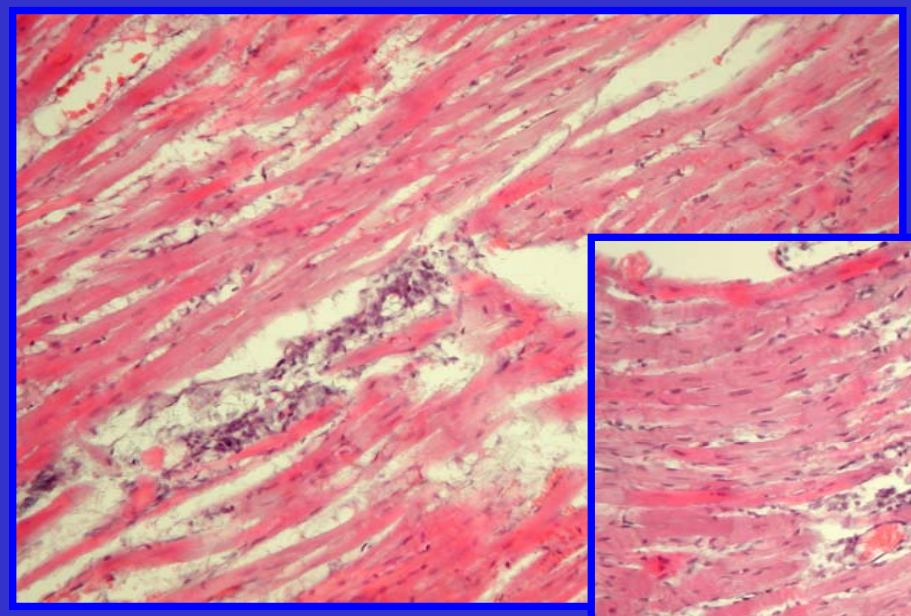


Long-term surviving animals post treatment with Dox-NP+Ps

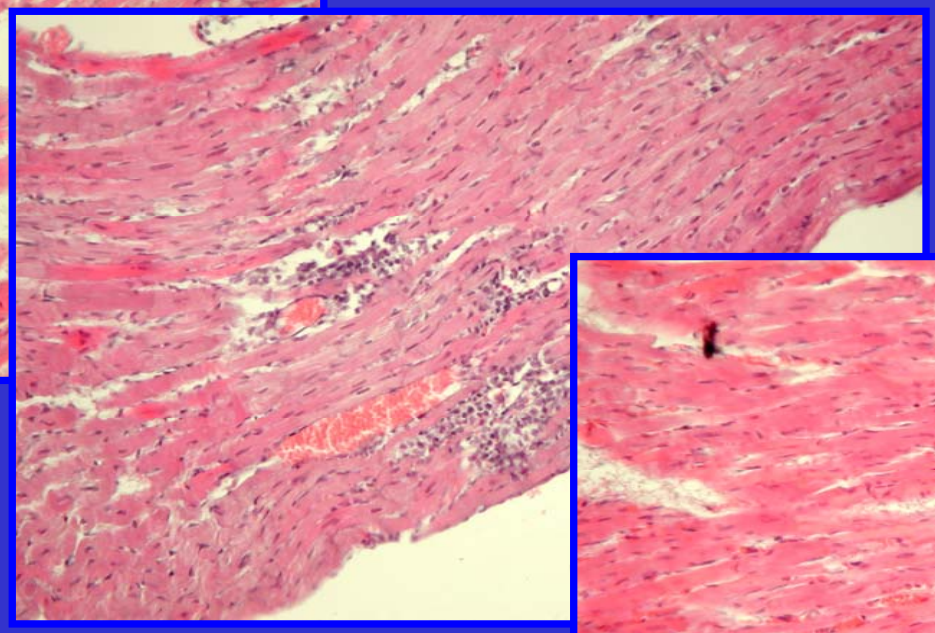
No evidence of neuronal apoptosis

Steiniger S. Int. J. Cancer 109 (2004) 759 - 767.

Histology: Myocardium

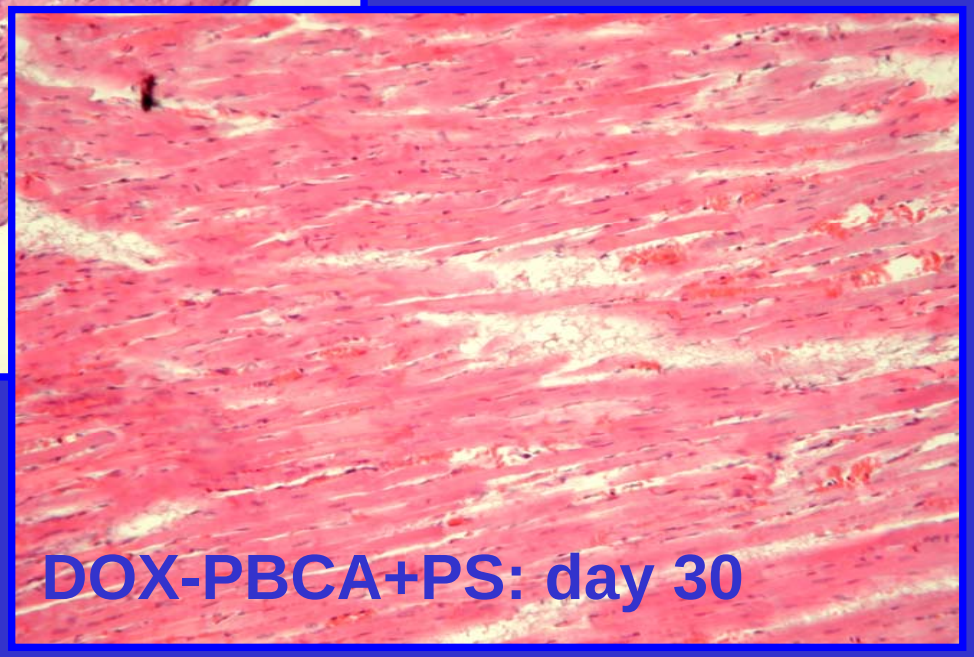


DOX: day 30



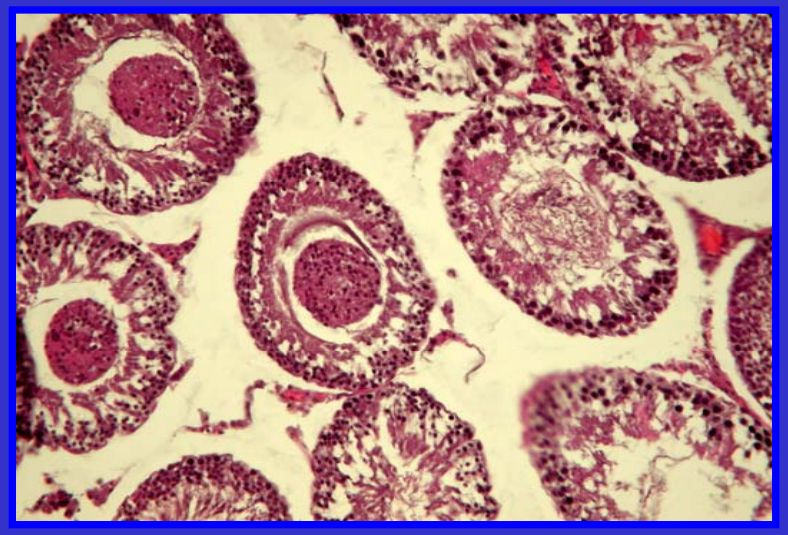
DOX-PBCA: day 30

Control

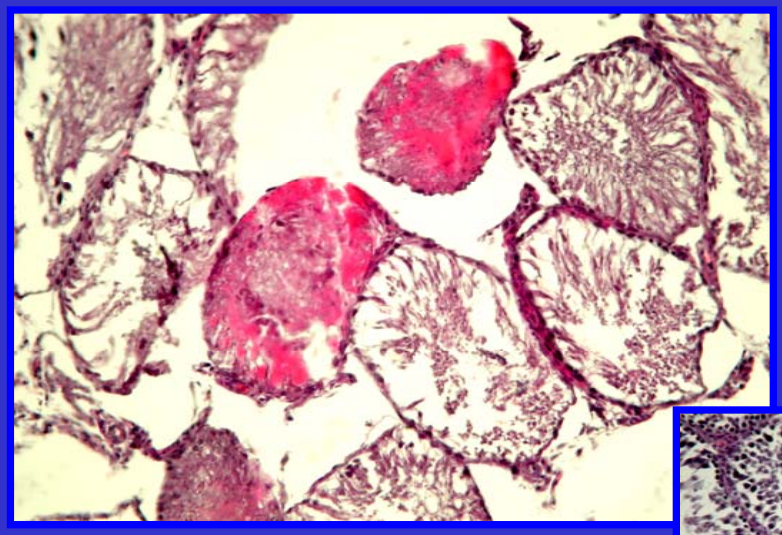


DOX-PBCA+PS: day 30

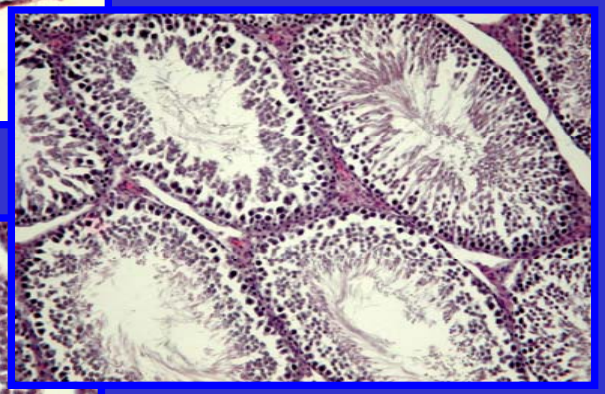
Histology: Testes



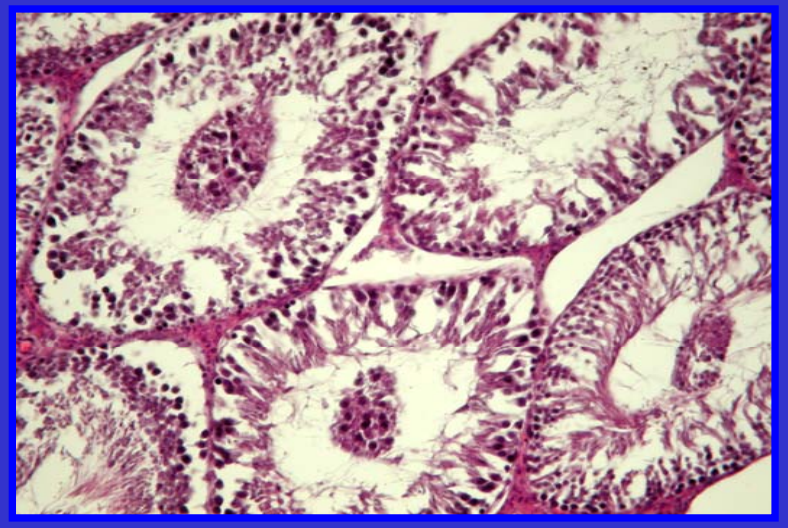
DOX - day 15



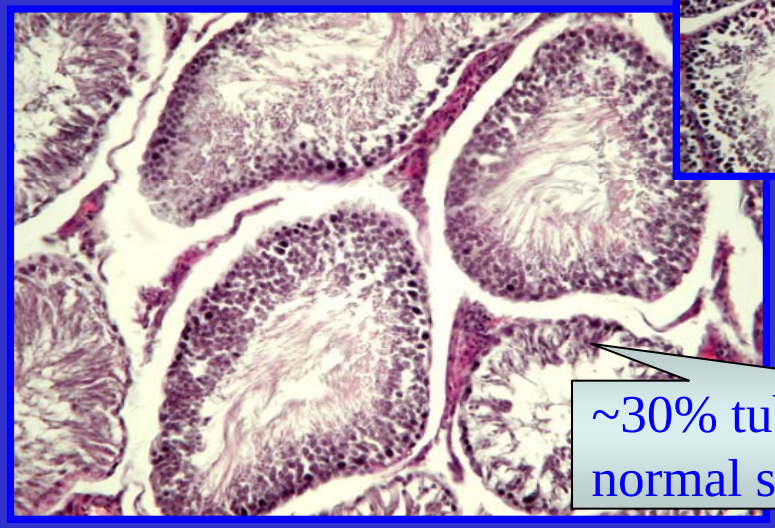
DOX – day 30



Control



DPBCA+Ps80 - day 15



DOX-PBCA+Ps80 - day 30

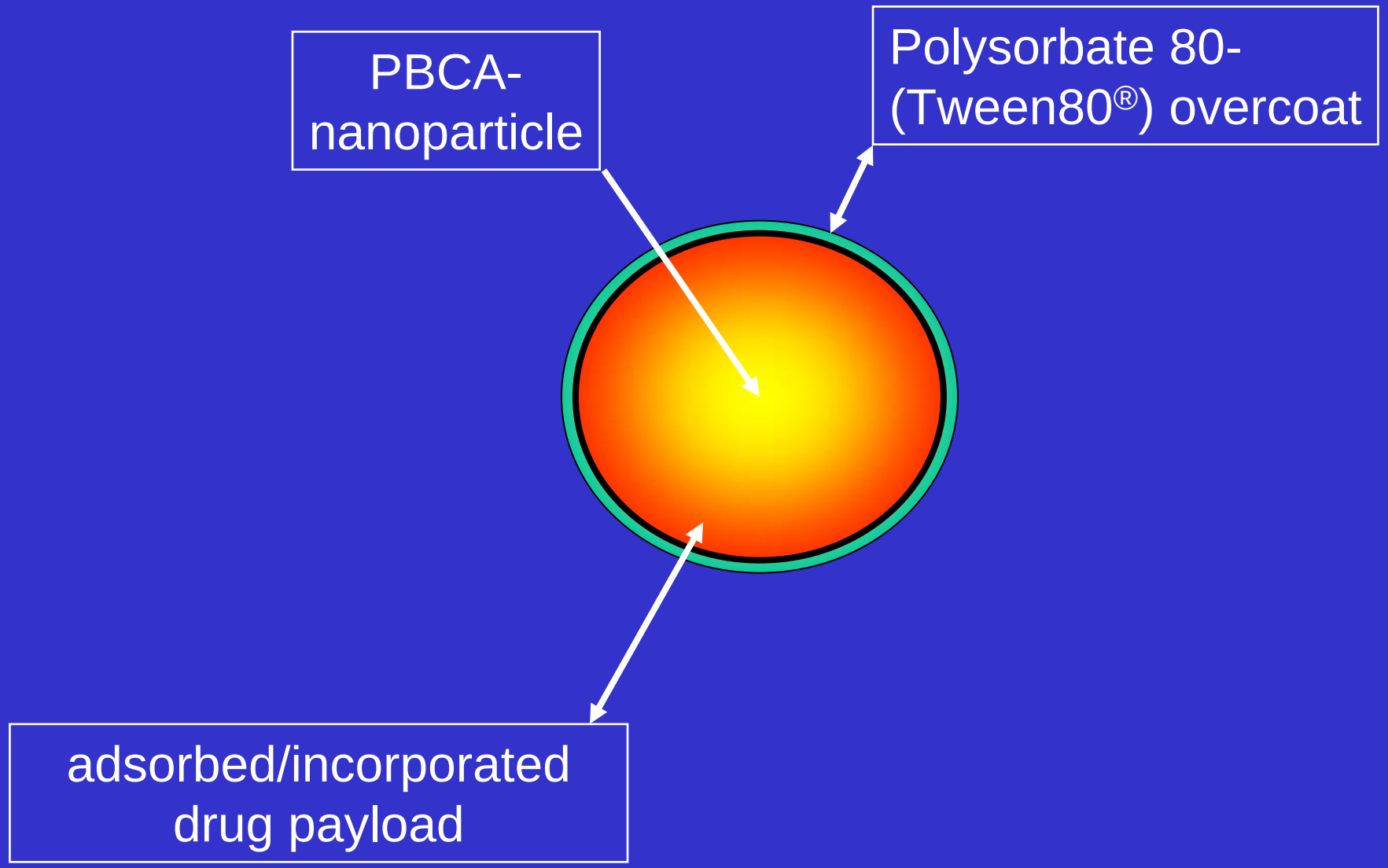
~30% tubules displayed normal spermatogenesis

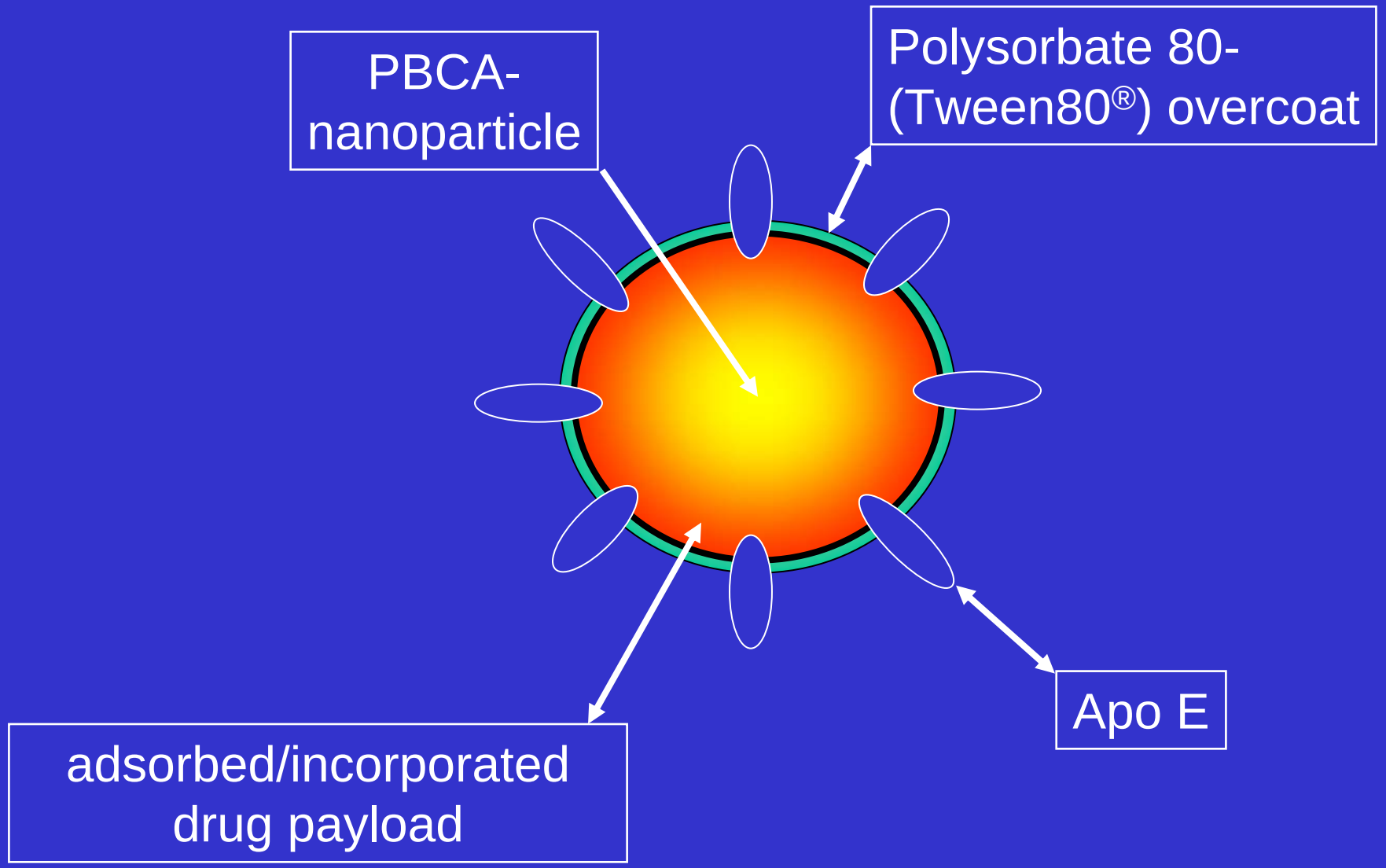
Mechanism of Nanoparticle Transport Across the BBB Using Nanoparticles

1. A general toxic effect
2. Inhibition of Pgp
3. Enhanced diffusion through adsorption at the luminal surface of the endothelial cells
4. General fluidisation of the endothelial cells by surfactants
5. Opening of the tight junctions
6. Endocytosis
7. Transcytosis
8. A combination of above mechanisms

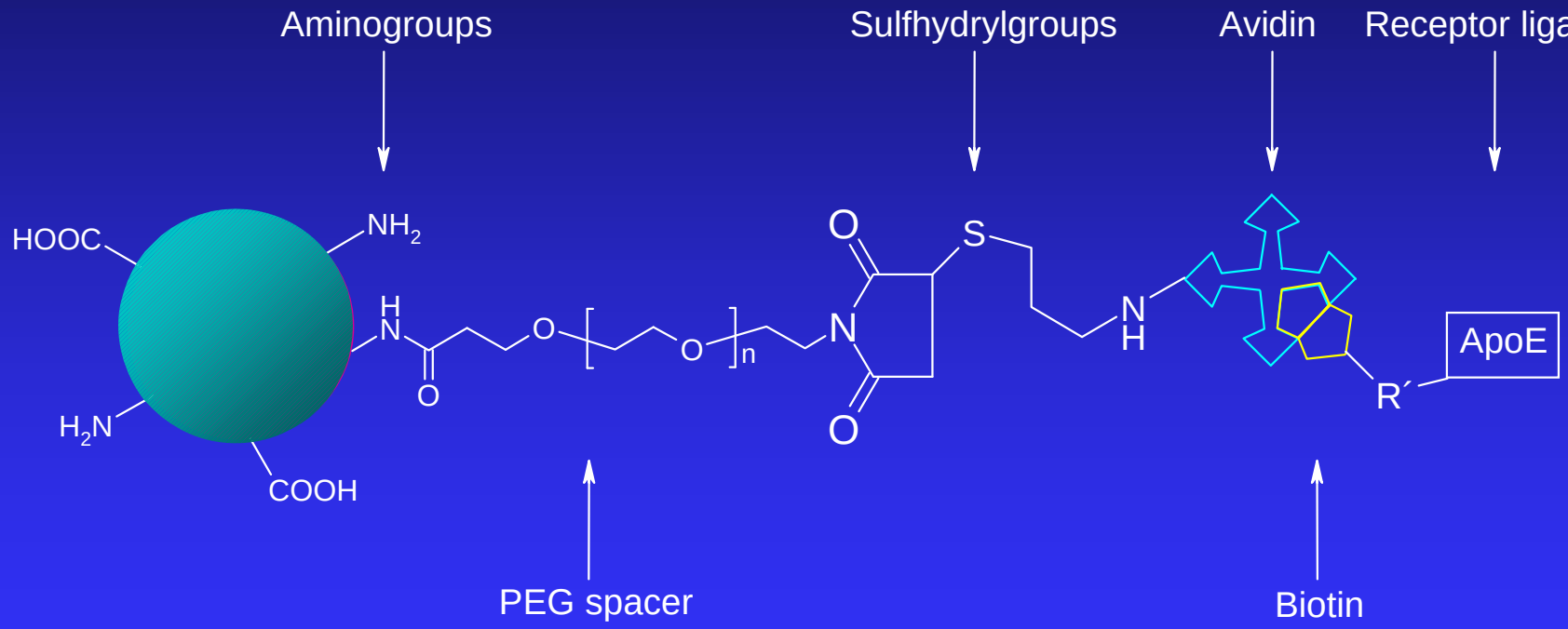
Mechanism of Nanoparticle Transport Across the BBB Using Nanoparticles

Endocytosis/Transcytosis

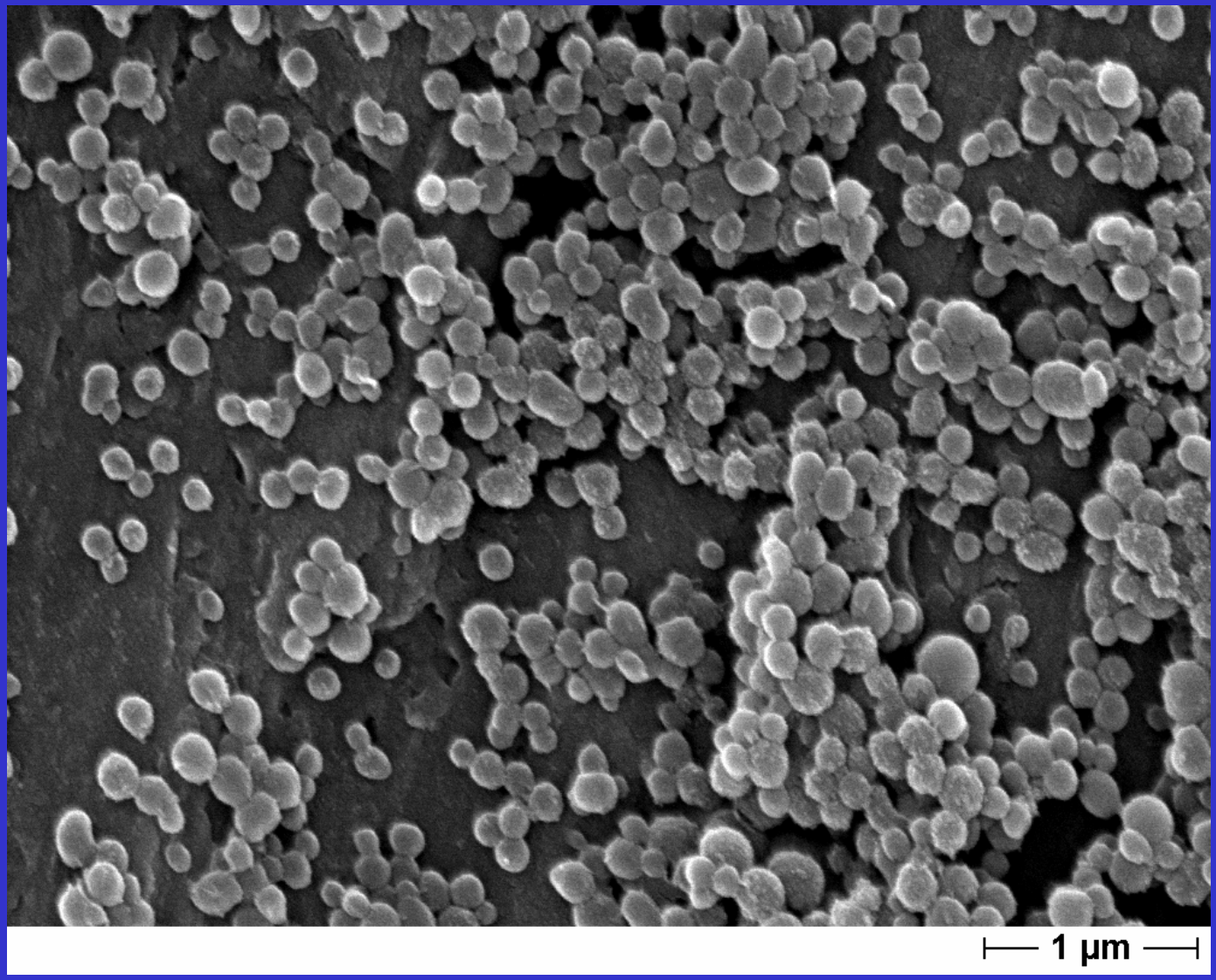

PBCA-nanoparticle
Polysorbate 80-(Tween80®) overcoat
adsorbed/incorporated drug payload


PBCA-nanoparticle
Polysorbate 80-(Tween80®) overcoat
Apo E
adsorbed/incorporated drug payload

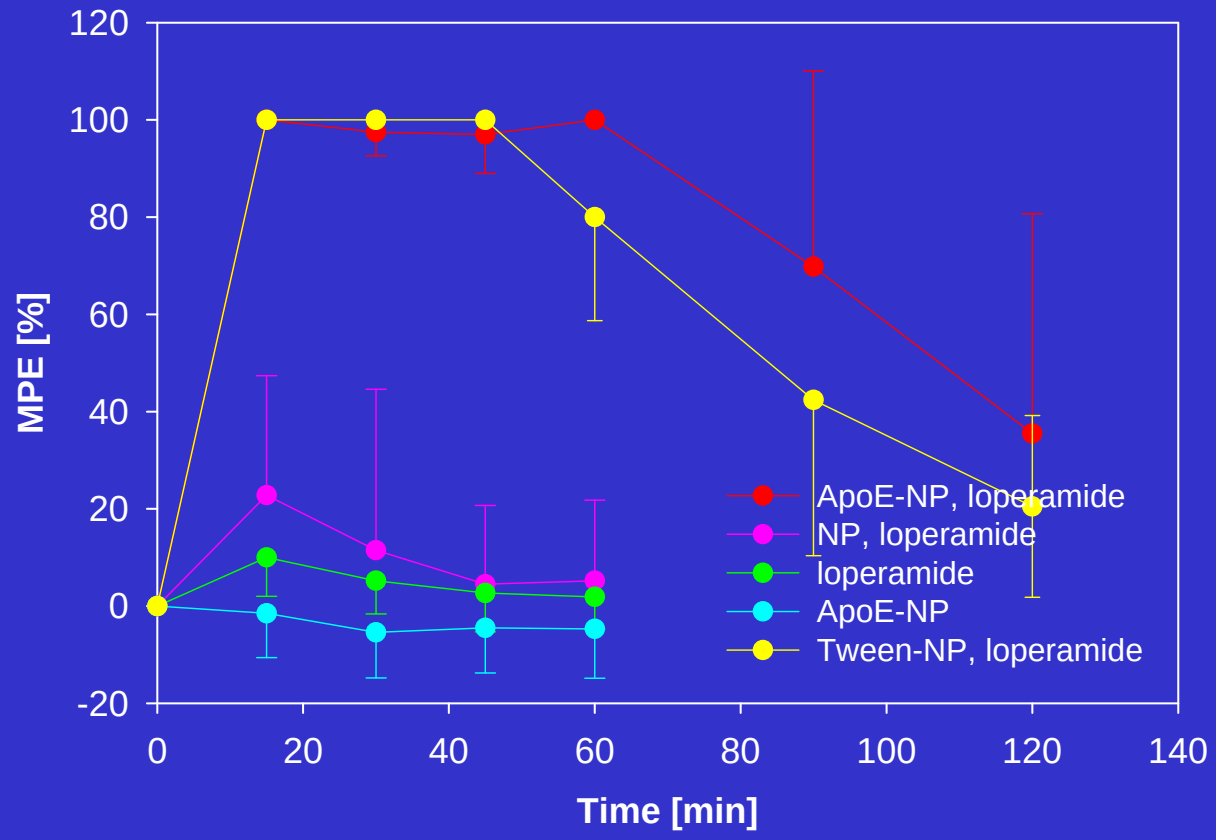
- Schematic structure of ApoE-modified NeutrAvidin-PEG-nanoparticles



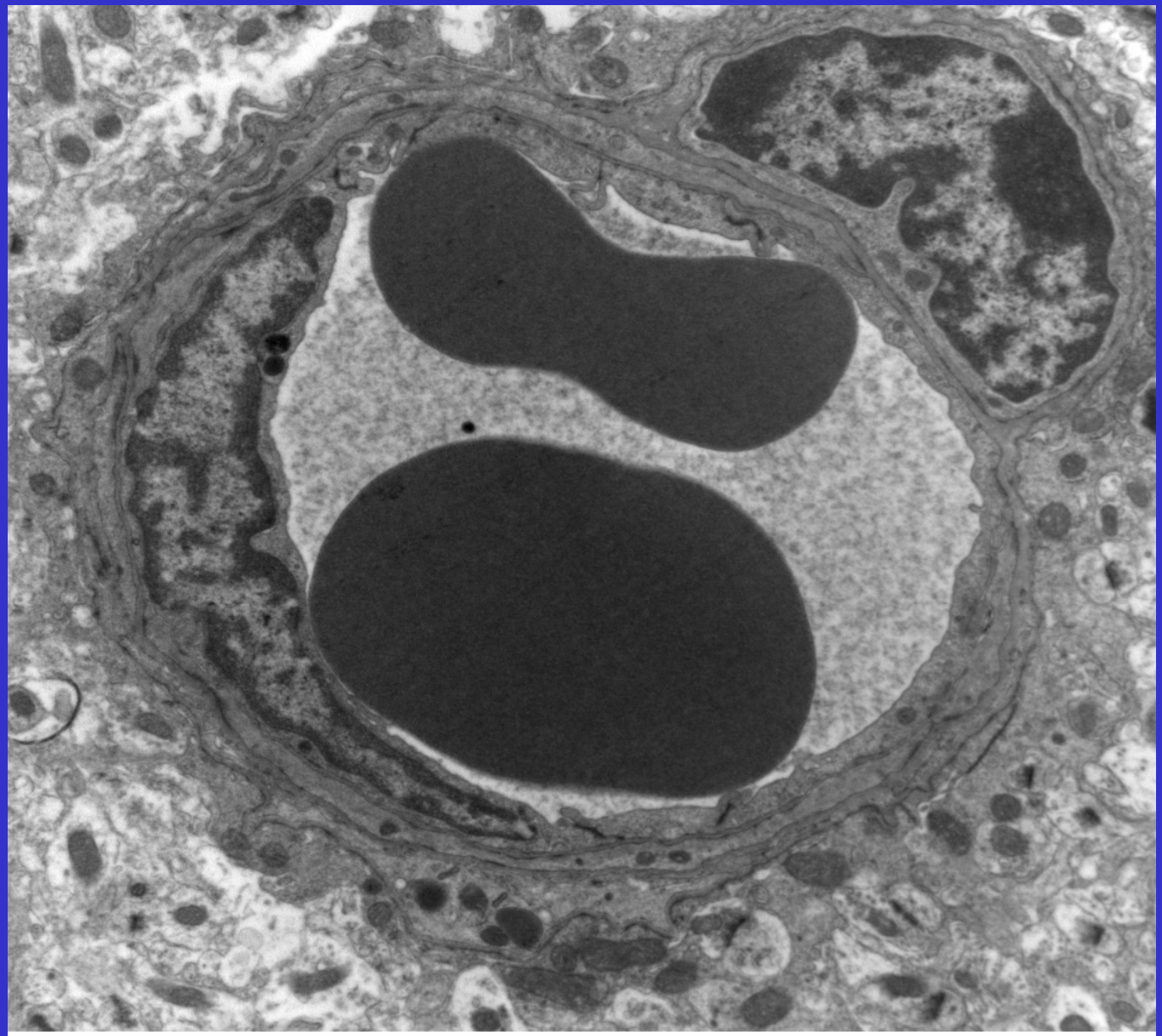
Human Serum Albumin Nanoparticles



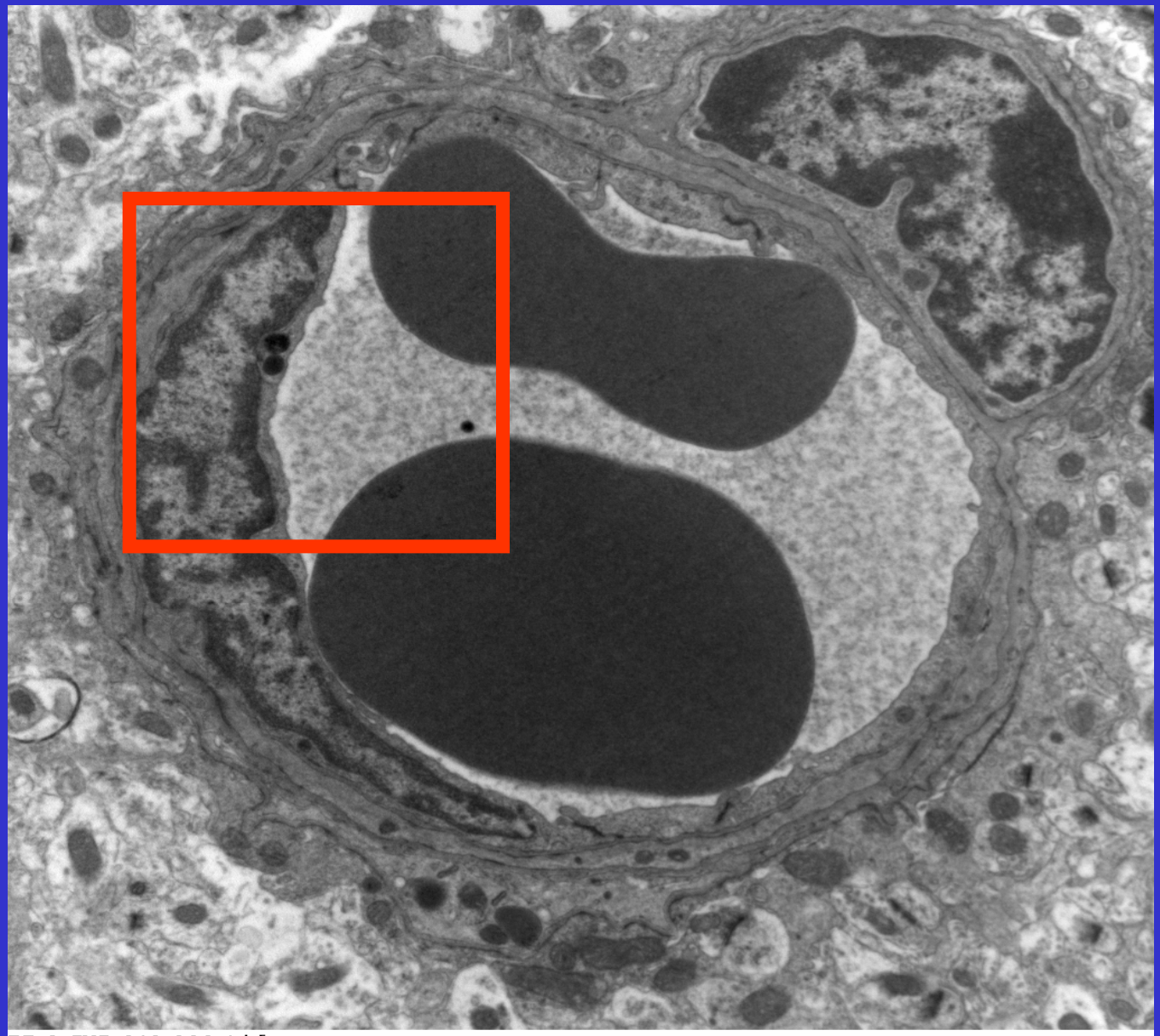
Analgesic effect of loperamid-loaded albumin-ApoE-NP



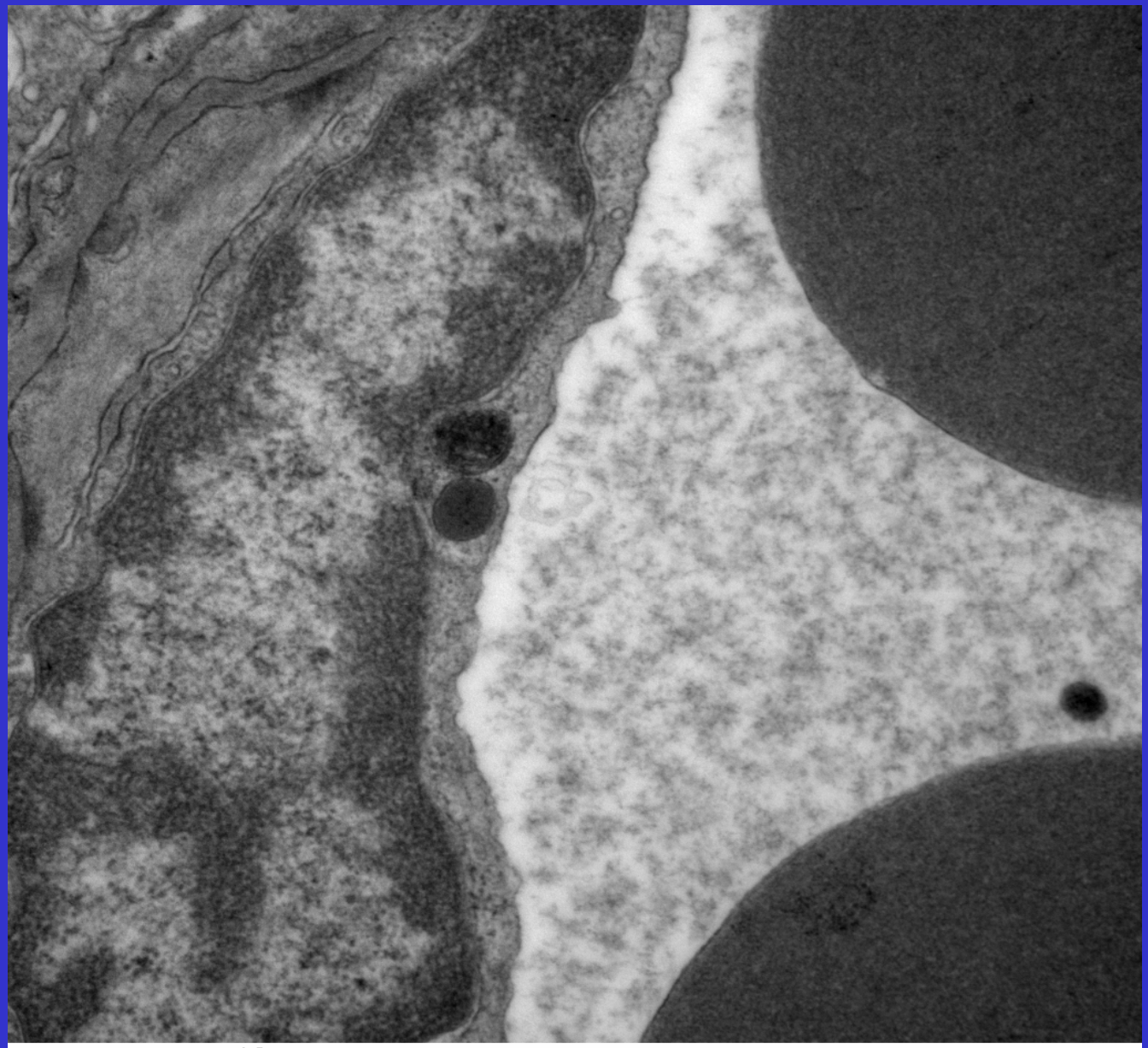
Transmisssion electron microscopical picture of a brain cortex blood vessel of a SV 129 mouse 30 min after intravenous injection of albumin nanoparticles with covalently bound apolipoprotein E



Transmisssion electron microscopical picture of a brain cortex blood vessel of a SV 129 mouse 30 min after intravenous injection of albumin nanoparticles with covalently bound apolipoprotein E

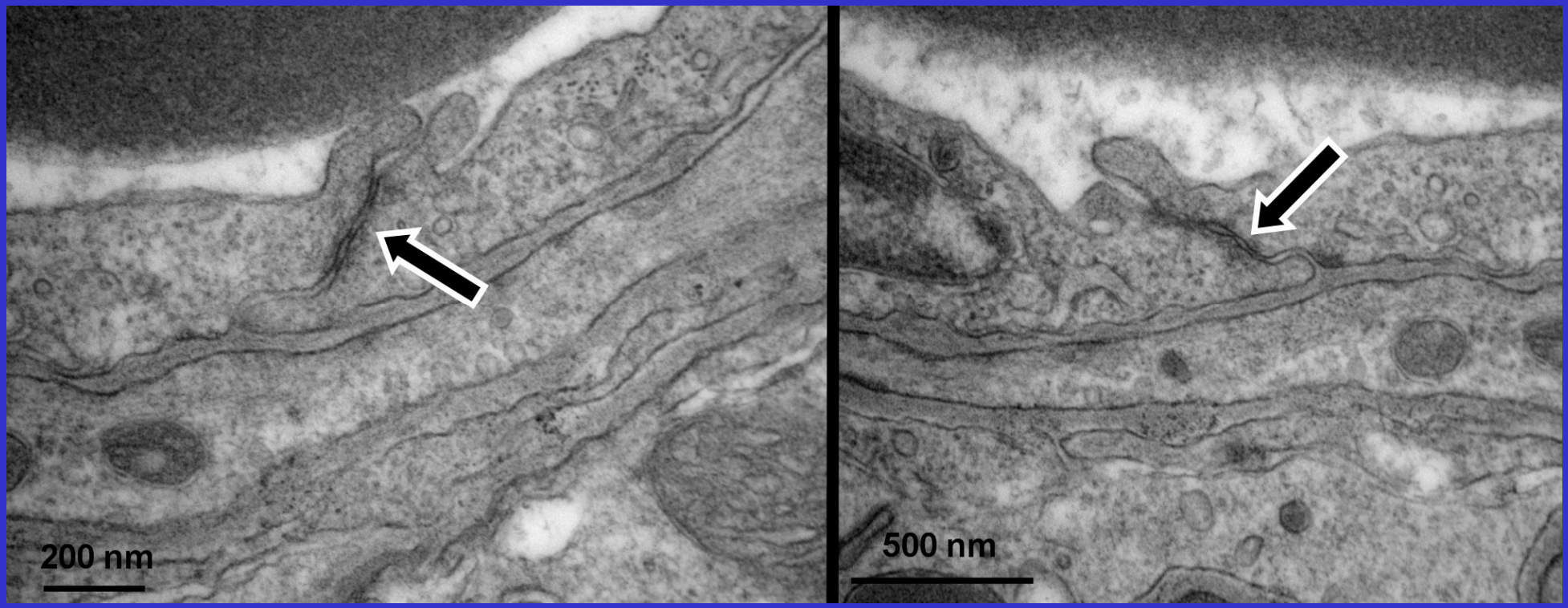


Transmisssion electron microscopical picture of a brain cortex blood vessel of a SV 129 mouse 30 min after intravenous injection of albumin nanoparticles with covalently bound apolipoprotein E

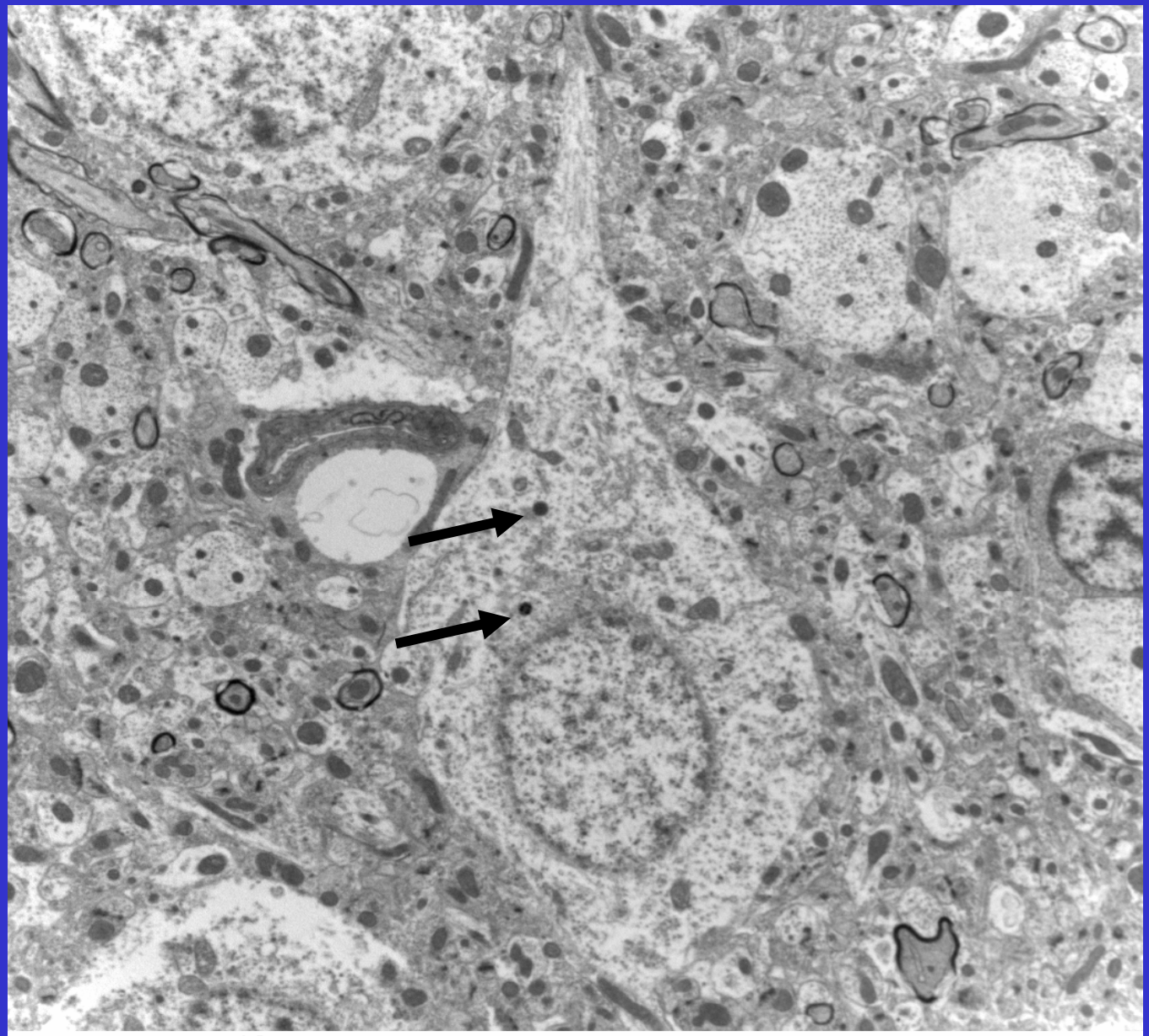


Co-injection experiments with lanthanum-III-nitrate showed:

- The tight junctions in the brain did not open.
- The nanoparticles were not transported through the tight junctions.

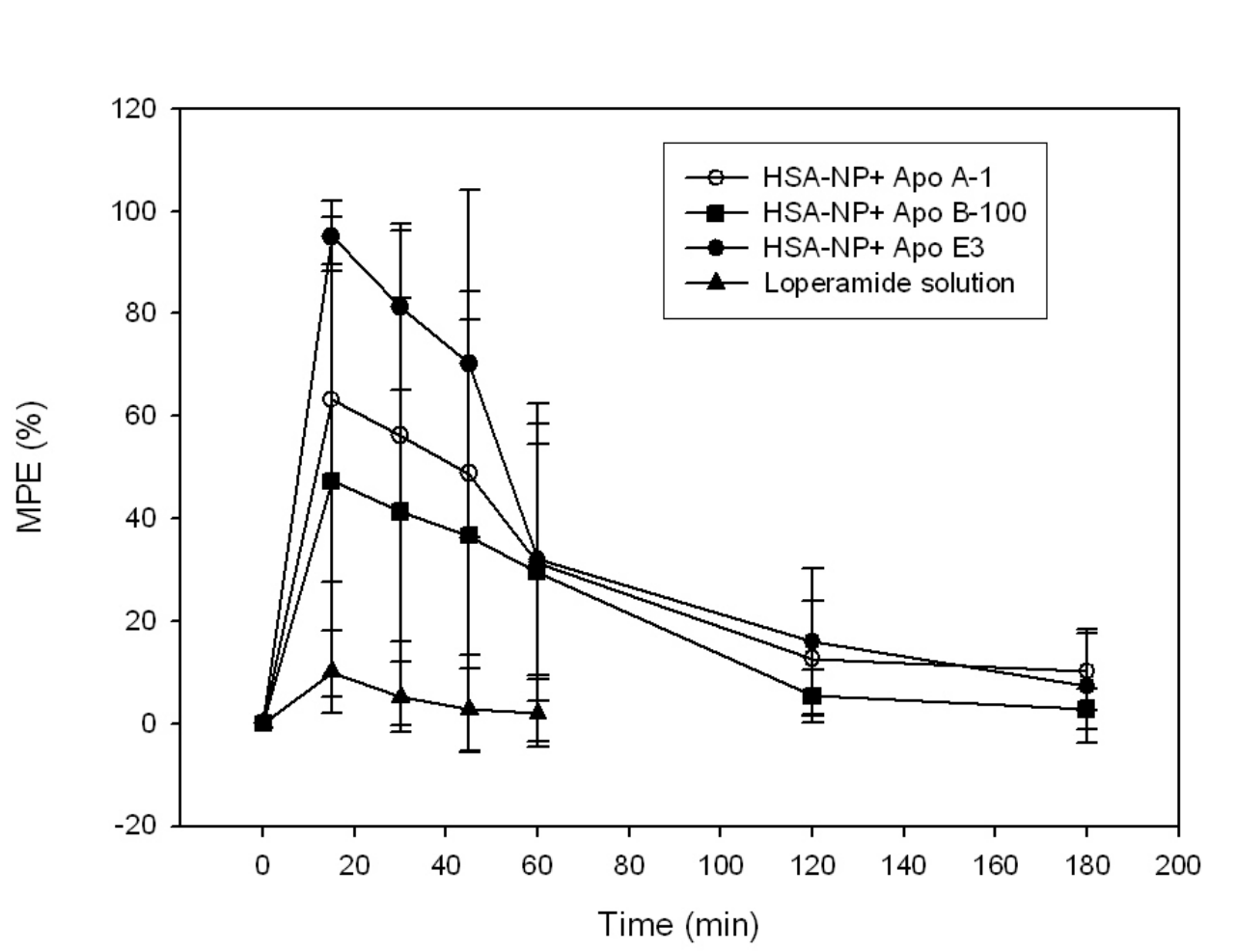


Transmisssion electron microscopical picture of a brain cortex neuron of a SV 129 mouse 30 min after intravenous injection of albumin nanoparticles with covalently bound apolipoprotein E



ATTACHMENT OF TARGETING LIGANDS

Analgesic Effect of Loperamid-Loaded HSA-Apo-A-1-NP and HSA-Apo-B-100 Nanoparticles



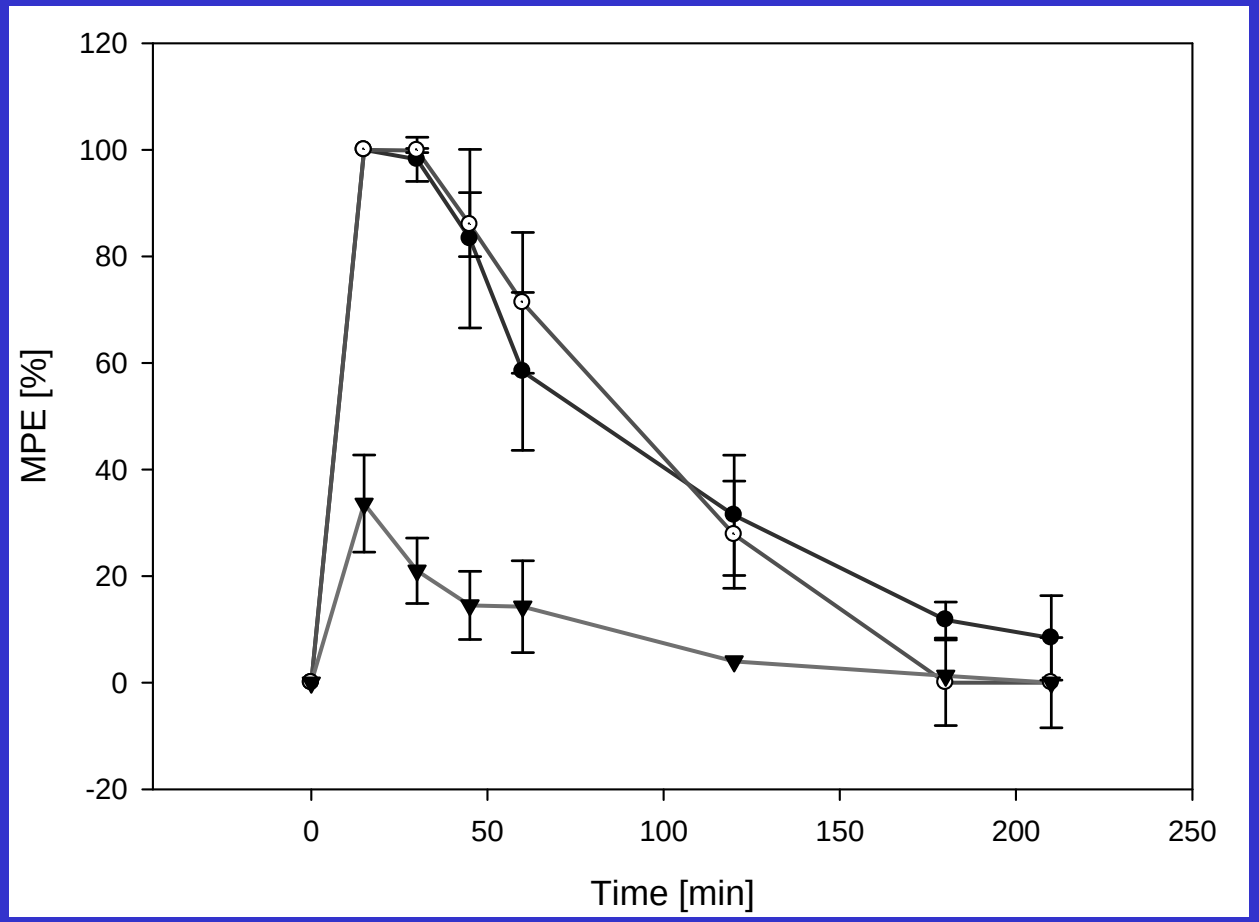
Analgesic Effect of Loperamid-Loaded Nanoparticles with Covalently Bound Anti-Transferrin-Antibodies

MPE of loperamide in mice after injection of nanoparticles with covalently attached antibodies:

Ox26 ○

R17217 ●

IgG2a ▼

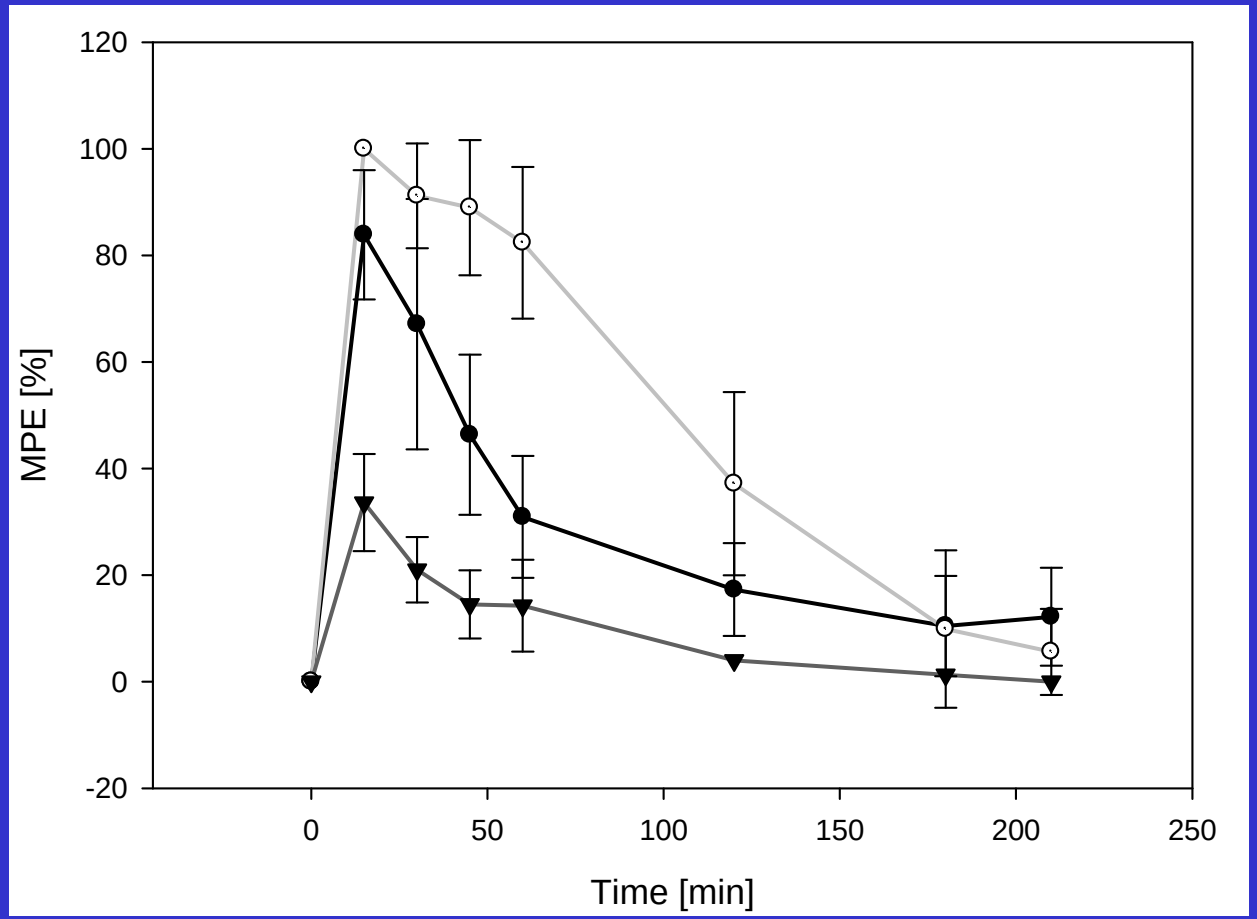


Analgesic Effect of Loperamid-Loaded Nanoparticles with Covalently Bound Transferrin

MPE of loperamide in mice after i.v. injection of NP with covalently attached transferrin (50.85-fold ●

or 76.2-fold molar excess ○ of 2-iminothiolane)

or with a covalently attached IgG2a antibodies ▼.



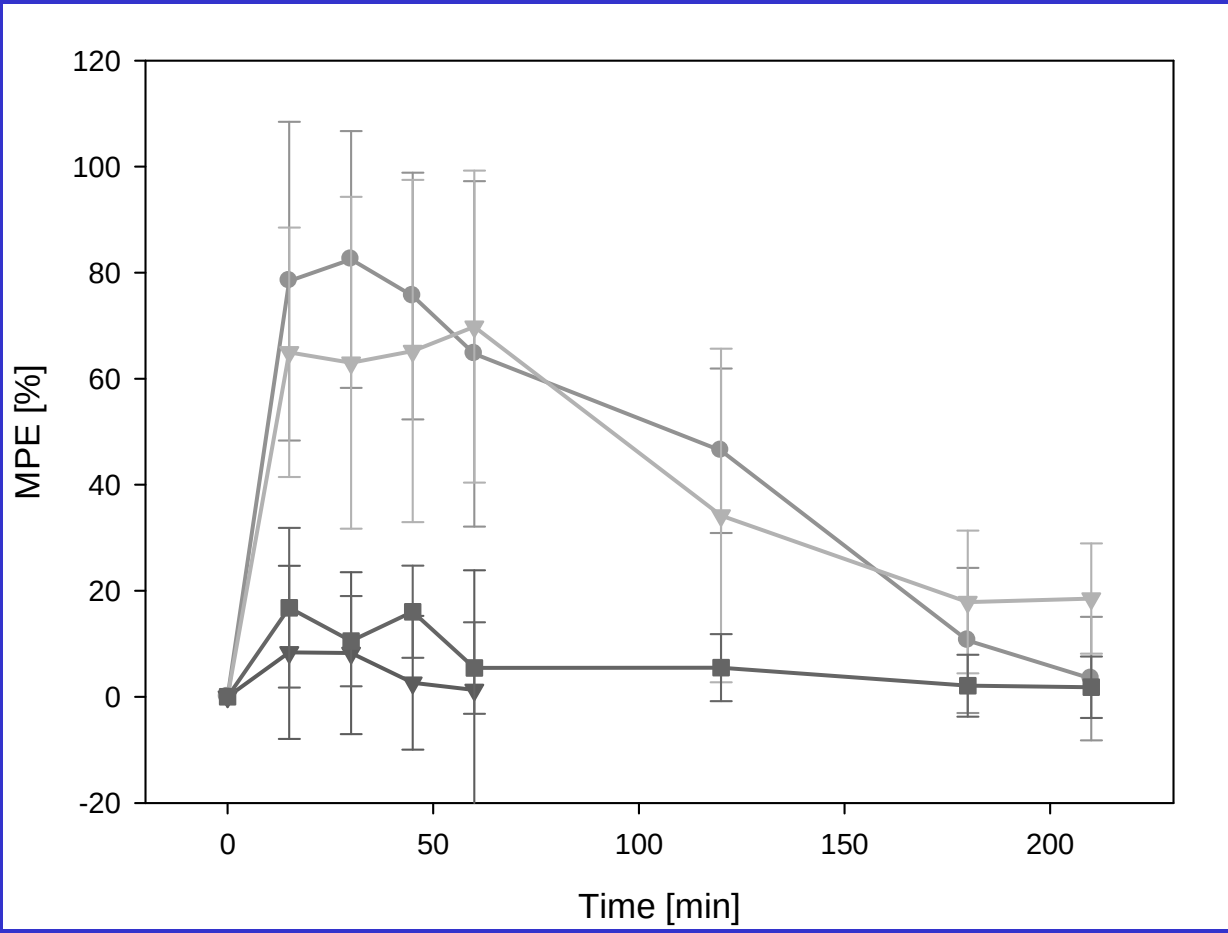
Analgesic Effect of Loperamid-Loaded Nanoparticles with Covalently Bound Insulin or Anti-Insulin-Antibodies

MPE in mice after i.v. injection of covalently attached IgG antibodies ■

Covalently attached 29B4 antibodies ● (grey)

Covalently attached insulin ▼ (grey)

Pre-injection of a 29B4 solution, 30 min before injection of NP with attached insulin ▼ (black)



COMMERCIALLY AVAILABLE NANOPARTICLES

• **Abraxane® Paclitaxel-loaded albumin NP**	**Abraxis Oncology, Los Angeles, USA**

CONCLUSIONS

- Polysorbate 80-coated nanoparticles (DOX-NP+PS) represent a very promising preparation for the delivery of drugs across the blood-brain barrier.
- A high incidence of tumour cure was observed in the extremely aggressive glioblastoma 101/8 with polysorbate 80-coated doxorubicin nanoparticles.
- Histologically there were no indications of neurotoxicity

ACKNOWLEDGEMENTS

- INTAS, Brussels
- Deutsche Forschungsgemeinschaft
- BMBF
- Familie Sutrup
- Sicor, Rho, Italy

ACKNOWLEDGEMENTS

SEBASTIAN STEINGER	J. W. Goethe-Universität Frankfurt
KLAUS LANGER	
KERSTIN MICHAELIS	
SEBASTIAN DREIS	
TELLI HEKMARTARA	
KATRIN D. GEIGER	J. W. Goethe-Univ. Z. Zt. Univ. Leipzig
HAGEN VON BRIESEN	Fraunhofer Inst. Biomed. Eng., St. Ingbert
SVETLANA GELPERINA	Nanosytem Ltd., Moscow
ALEXANDER S. KHALANSKY	Institute of Human Morphology, Moscow
RENAD N. ALYAUTDIN	Sechenov Medical Academy, Moscow
DAVID BEGLEY	King‘s College London
RAINER MÜLLER	Freie Universität Berlin