Supplementary Information

S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
SynB1	Benzylpenicillin	*In situ* brain perfusion in rats (single time) + capillary depletion	*In vivo*	Q: Without SynB1: K_{in} = 0.15 µl/s/g ↔ coupled to SynB1: K_{in} = 1.14 µl/s/g. 80% SynB1 coupled benzylpenicillin in parenchyma.	[1]
SynB1	Dalargin	*In situ* brain perfusion (single time) in rats + antinociceptive test	*In vivo*	Q: Without CPP: V_d = 16.7 µl/g ↔ coupled to SynB1: V_d = 309 µl/g. Enhancement of analgesic activity.	[2]
SynB1	Doxorubicin	*In situ* brain perfusion in rats (single time) + capillary depletion	*In vivo*	Q: Without CPP: K_{in} = 0.25 µl/s/g ↔ coupled to SynB1: K_{in} = 1.50 µl/s/g. 70% SynB1 coupled benzylpenicillin in parenchyma.	[3]
SynB1	Doxorubicin	*In situ* brain perfusion in mice (single time) + capillary depletion	*In vivo*	Q: Without CPP: V_d = < 100 µl/g ↔ coupled to SynB1: V_d = 776.4 µl/g. 60% SynB1 coupled doxorubicin in parenchyma.	[4]
SynB1	PEG-gelatin-siloxane nanoparticles	Determination transcellular transport across co-culture BBB model and determination of PG-GS-SynB particles in brain after IV injection of mice using *in vivo* imaging.	*In vitro/ In vivo*	D: Vectorizing the nanoparticles with the SynB peptide enhances the transport across the BBB *in vitro* as well as *in vivo*.	[18]
SynB3	Dalargin	*In situ* brain perfusion (single time) in rats + antinociceptive test	*In vivo*	Q: Without CPP: V_d = 16.7 µl/g ↔ coupled to SynB3: V_d = 240 µl/g. Enhancement of analgesic activity.	[2]
(L- and D-) SynB3	Doxorubicin	*In situ* brain perfusion in mice (single time) + capillary depletion	*In vivo*	Q: Without CPP: V_d = < 100 µl/g ↔ coupled to (L-en D-) SynB3: V_d = 961.8 (L) and 788.4 (D) µl/g. 50% SynB1 coupled doxorubicin in parenchyma.	[4]
SynB3	Paclitaxel	*In situ* brain perfusion in mice	*In vivo*	D: Vectorized paclitaxel bypasses Pgp at luminal side of BBB.	[5]
SynB3	Morphine-6-glucuronide	*In situ* brain perfusion in mice + antinociceptive tests	*In vivo*	Q: Without CPP: K_{in} = 0.024 µl/s/g ↔ coupled to SynB3: K_{in} = 1.27 µl/s/g. Improvement of pharmacological activity.	[6]

Supplementary Information

S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
SynB3	Endomorphin-1 (linked by disulfide linkage)	Tail flick antinociceptive test	*In vivo*	Q: Five-fold increase in antinociception compared to EM-1 alone.	[39]
D-Penetratin	Doxorubicin	*In situ* brain perfusion in rats (single time) + capillary depletion.	*In vivo*	Q: Without CPP: K_{in} = 0.25 µl/s/g↔ coupled to D-Penetratin: K_{in} = 2.14 µl/s/g. 70% SynB1 coupled benzylpenicillin in parenchyma.	[3]
Penetratin	-	IV injection → stained with fluorescent tag.	*In vivo*	D: no staining → no brain influx.	[7]
Penetratin	scFvs	Mice were IV injected with scFv-CPP construct. Then the presence of scFv-CPP in brain cryosections was evaluated after fluorescent labeling using a fluorescence microscope.	*In vivo*	D: scFv-CPP construct clearly appeared in brain cells after IV injection.	[26]
Penetratin	Doxorubicin loaded transferrin liposomes	The amount of doxorubicin in brain homogenate was evaluated using HPLC at different time points after IV injection in rats.	*In vivo*	Q: Tf-Penetratin liposomes showed maximal brain penetration after 24h (about 3.67% ID/g).	[27]
Penetratin	PEG-PLA nanoparticles loaded with coumarin-6	*In vivo* imaging and pharmacokinetic and biodistribution studies using LC-MS/MS analysis of coumarin-6 in brain homogenate.	*In vivo*	D: Fluorescence in rat brain was higher for penetratin-NP treated rats than for NP-treated ones. Brain uptake was enhanced when NP was coupled to penetratin.	[28]
Tat 47-57	NEP1-40	Focal ischemia model in rats → evaluate outcome after ischemia + detect presence of vectorized NEP1-40.	*In vivo*	B: Improvement of neurologic outcomes. D: Tat-NEP1-40 detected in brain using Western blot and immunofluorescence.	[8]

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S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
Tat 47-57	NR2B9c	Measuring effect of vectorized NR2B9c in a rat stroke model.	*In vivo*	Q: Treatment with Tat-NR2B9c reduced the volume of cerebral infarction with 67% and 87% in cortical infarction volume.	[10]
Tat 47-57	β-galactosidase	Fluorescence confocal microscopy: tissues were dissected from mice 20 min after i.p. injection	*In vivo*	D: - Tat-FITC: strong signals in all areas of brain (not with control FITC). - Tat-β-Gal: brain sections from mice analyzed: 2h post injection, strong activity around capillaries, not in parenchyma, starting from 4h after injection all brain regions showed strong β-Gal activity. - BBB remained intact (Evan's blue albumin complexes not in brain sections).	[11]
Tat 47-57	Green fluorescent protein (GFP)	Evaluation of transcellular transport of Tat-GFP across bEnd-3-astrocyte co-culture layer.	*In vitro*	D: Tat-GFP was able to translocate bEnd-3 cell layer but not astrocyte layer. No influence on barrier integrity observed.	[12]
Tat 47-57	PEG-b-Chol nanoparticles (loaded with ciprofloxacine, quantum dots or FITC)	Confocal microscopy of rat brain sections 4h after IV injection in the tail vein.	*In vivo*	D: Tat-conjugated nanoparticles loaded with FITC or quantum dots crossed the BBB, while FITC and quantum dots alone did not and were localized around blood vessels in the brain.	[13]
Tat 47-57	PEG decorated gelatin-siloxane nanoparticles	*In vivo* imaging (mice) and TEM.	*In vivo*	Q: Tat-modification of the nanoparticles resulted in a quantitatively higher fluorescent signal in the brain than no-Tat nanoparticles (total signal counts of 708.69 ± 4.8 counts/ (sc × s) versus 670.47 ± 8.96 counts/ (sc × s)). TEM analysis revealed that the BBB remained intact.	[14]
Tat 47-57	δ-V1-1 (isozyme specific inhibitor of δ-PKC)	Two *in vivo* models of vascular stress: transient focal ischemia in normotensive rats and chronic hypertension.	*In vivo*	B: δ-V1-1-Tat increased the number of patent microvessels by 92% compared to control (Tat) treated animals and increased cerebral blood flow by 26% following acute focal ischemia (not with Tat peptide alone). In chronic hypertension model, the cerebral blood flow increased by 12%.	[15]

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S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
Tat 47-57	PACAP-38	Six hours after i.p. injection of FITC-labeled peptides, the fluorescence intensity of isolated brains was determined.	*In vivo*	B: PACAP-Tat showed a 2.5 fold higher efficiency to traverse the BBB than PACAP.	[16]
Tat 47-57	GDNF	Evaluation of cryosections of mouse brain 4h post i.p. injection using fluorescence microscopy.	*In vivo*	D: The fusion proteins crossed the BBB and transduced the brain parenchyma.	[17]
Tat 47-57	PEG-cholesterol (PEG-b-Col) nanoparticles	Evaluation of presence of FITC loaded PEG-b-Col-Tat nanoparticles in brain cryosections using confocal microscope 4h after IV injection of rats.	*In vivo*	D: PEG-b-Col-Tat particles crossed the BBB.	[19]
Tat 47-57	Bcl-X_L	Evaluation of infarct volume and neurological deficit in ischemic insult mice model.	*In vivo*	D: Tat- Bcl-X_L reduces the infarct volume and neurological deficits when administered before and after ischemic insult.	[22]
Tat 47-57	GDNF	Evaluation whether IV administration of Tat-GDNF prevent brain injury after transient focal ischemia.	*In vivo*	D: After IV administration, the Tat-GDNF protein reaches the ischemic zone and reduced the brain injury and infarction zone.	[23]
Tat 47-57	Ritonavir loaded nanoparticles	Measuring radioactivity of ritonavir in brain tissue digest after decapitation of mice at different time points.	*In vivo*	Q: The brain drug level was 800-fold higher than that with drug in solution at two weeks (0.1 μg/g (solution) versus 80.3 μg/g (Tat-nanoparticles)).	[25]
Tat	Doxorubicin loaded transferrin ligated liposomes	The amount of doxorubicin in brain homogenate was evaluated using HPLC at different time points after IV injection in rats.	*In vivo*	Q: Tf-Penetratin liposomes showed max brain penetration after 24h (about 2.89% ID/g).	[27]

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S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
Tat	Quantum dots	Rats were infused intra-arterially and after euthanizing the rat, the brain was isolated and quantum dot fluorescence was evaluated.	*In vivo*	D: If quantum dots are conjugated to Tat, the brain tissue was labeled. Histological data confirm the passage across the endothelial cell line of the blood-brain barrier.	[31]
D-Tat	^{99m}Tc-Tricarbonyl and fluorescein-5-maleimide	Biodistribution was evaluated in mice. At several time points after IV injection, tissues were evaluated by fluorescence microscopy and radiometric analysis.	*In vivo*	D: Little brain permeation was determined.	[32]
Tat	G_3R_6-cholesterol (conjugated to Tat and forms nanoparticles)	4h after i.v. injection of the nanoparticles, rabbit brain sections were evaluated for FITC-loaded nanoparticles using a confocal microscope.	*In vivo*	D: FITC was detected in the brain sections when coupled to CG_3R_6TAT nanoparticles (not if not coupled to nanoparticles), indicating the nanoparticles cross the BBB.	[34]
Tat 47-57	Cholesterol liposomes loaded with coumarin-6	After i.v. injection of the coumarin-6 loaded TAT-liposomes in mice, the coumarin-6 concentration was determined in brain tissue.	*In vivo*	D: The AUC (0-t) for TAT-liposomes was 1.79 to 2.54 times higher than non-conjugated liposomes.	[35]

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S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
(D-)Tat 47-57	Liposomes	*In vivo* biodistribution study in mice of (D-)Tat-coupled liposomes loaded with coumarin-6. At different time points after IV injection, the concentration of coumarin-6 was determined *i.a.* in brain tissue. Capillary depletion study was used to demonstrate distribution to brain parenchyma.	*In vivo*	Q: The concentration of coumarin-6 delivered using (D-)Tat-coupled liposomes was 2 to 2.5 times higher than liposomes coupled to a Tat analogue with basic residues replaced by glycine and glutamic acid residues.	[36]
Tat 48-57	FITC doped silica nanoparticles (FSNPs)	The Tat-FSNPs were intra-arterially injected into rats. After completing the procedure, the rats were decapitated and the brain was sliced into 4 pieces and imaged using a fluorescence microscope.	*In vivo*	D: The images confirm labeling of branches of the right middle cerebral artery. Thus, not the nanoparticles crossed the blood-brain barrier.	[37]
Tat 46-57	Doxorubicin loaded nanoparticles (and co-modified with T7 ligand)	*In vivo* imaging and evaluation of survival time of tumor bearing mice.	*In vivo*	D/B: Compared to control liposomes, the TAT-T7 co-modified doxorubicin-loaded liposomes markedly accumulated in the glioma brain tumor. Also the survival time of these mice significantly increased.	[40]
Tat 47-57	VIP	Efficiency assay of traversing the BBB by fluorimetry; food intake assay and evaluation of effect on scopolamine induced amnesia.	*In vivo*	Q: After i.p. injection, the brain uptake efficiency of VIP-TAT (1.81) was twice as high as that of VIP (0.78). B: VIP-TAT had a significantly stronger anorexigenic effect than VIP. Q/B: Administration of VIP-TAT significantly inhibited stronger than VIP alone the reduction of the latent time induced by scopolamine.	[41]

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S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
Tat 47-57	BRBP1 (and linked to the proapoptotic peptide KLA as well)	IV injection of fluorescently labeled BRBP1-TAT-KLA in mice with breast cancer brain metastasis.	*In vivo*	D: Compared to TAT-KLA, BRBP1-TAT-KLA showed higher fluorescence intensity in the brain metastasis lesions.	[44]
R7-myristoylated	-	NIR fluorescence imaging after IV injection + immunohistochemical staining.	*In vivo*	D: Presence of fluorescently labeled vector demonstrated.	[9]
rR9	RVG29-cargo (plasmid DNA)	Evaluation of luciferase activity of in several tissues after injection of rR9-RVG29-pGL3 (gene) construct in the tail vein of mice.	*In vivo*	B: At 72h, the luciferase expression was 3-fold higher than for the control group.	[21]
R8	RGD	Translocation across an *in vitro* BBB model AND *in vivo* imaging in C6 glioma bearing mouse model.	*In vitro/ In vivo*	D: R8-RGD reached the brain and accumulated in the glioma foci. Also *in vitro*, R8-RGD crossed the BBB.	[24]
R8	Liposomes	*In vivo* biodistribution study of R8-coupled liposomes loaded with coumarin-6. At different time points after IV injection, the concentration of coumarin-6 was determined *i.a.* in brain tissue. Capillary depletion study was used to demonstrate distribution to brain parenchyma.	*In vivo*	Q: The concentration of coumarin-6 delivered using R8-coupled liposomes was 3.5 times higher than liposomes coupled to a Tat analogue with basic residues replaced by glycine and glutamic acid residues.	[36]
R2-R5	EM-1 analogs	Antinociceptive test	*In vivo*	D: Unless the decreased affinity for the opioid receptor, the vectorized analogs showed potent antinociceptive effect, partly caused by the improved bioavailability.	[29]

Supplementary Information

S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
Oligo-arginine	Transferrin ligated liposomes	Rat were IV injected with vectorized liposomes and at different time points, brain was isolated and evaluated using NIR imaging.	*In vivo*	D: The brain penetration of Tf-PR-liposomes was 8-fold greater than plain liposomes.	[30]
R11	No cargo, but FITC labeled.	Evaluation of immunoreactivity (using goat anti-FITC antibodies) in mouse brain sections after systemic administration of 11R-FITC.	*In vivo*	D: Strong immunoreactivity was observed in vessels and surrounding cells of cortex, striatum and thalamus, which was not seen when systemically injecting 11E-FITC.	[43]
pVEC	pVEC covalently attached to gHo (glioma homing sequence) = gHoPe2	Evaluation of presence of FAM-labeled gHoPe2, IV injected in mice, in cryosections of the brain.	*In vivo*	D: In intracranial brain tumor model, the gHoPe2-FAM peptides were present in the intracranial tumors, not in healthy brain tissue. Thus the construct crossed the BBB.	[20]
Mastoparan	Doxorubicin loaded transferrin liposomes	The amount of doxorubicin in brain homogenate was evaluated using HPLC at different time points after IV injection in rats.	*In vivo*	D: Accumulation of Tf-Mastoparan liposomes was lower compared to Tf-Tat and Tf-Penetratin liposomes.	[27]
(RXRRBR)2XB	AMO (antisense morpholino oligonucleotides)	IV injection to mice (single time and multiple time injection) and evaluation of presence of fluorescence in different brain areas using fluorescence microscopy.	*In vivo*	D: Fluorescence was widely detected throughout the brain and increased when multiple injections were given.	[33]
PepFect 32 (PepFect 14-Angiopep-2 construct)	pDNA	*In vitro* Transwell experiment. Transport across the bEnd.3 layer was demonstrated by measuring plasmid transfection in U87 cells.	*In vitro*	Q: PepFect 32 showed the most efficient transfection of the luciferase-expressing plasmid in U87 cells.	[38]

Supplementary Information

S1 Table. Overview literature studies describing brain delivery by cell-penetrating peptides.

CPP	Cargo	Technique	*In vivo/ in vitro*	(Quantitative) result*	Reference
LNP	pDNA	Evaluation of *in vitro* transport of LNP-modified pDNA nanoparticles across the BMEC layer.	*In vitro*	Q: P_{app} of LNP-modified pDNA nanoparticles was 92.43×10^{-6} cm/s, while if not LNP-modified, the P_{app} was $\leq 65 \times 10^{-6}$ cm/s.	[42]

* Q: Quantitative result; B: biological effect; D: effect described.

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Remarks:

Ref [7]: Penetratin (directly injected in brain) cause in a dose > 1 µg neurotoxic cell death and recruitment of inflammatory cells. At doses < 1 µg less pronounced toxic effect.