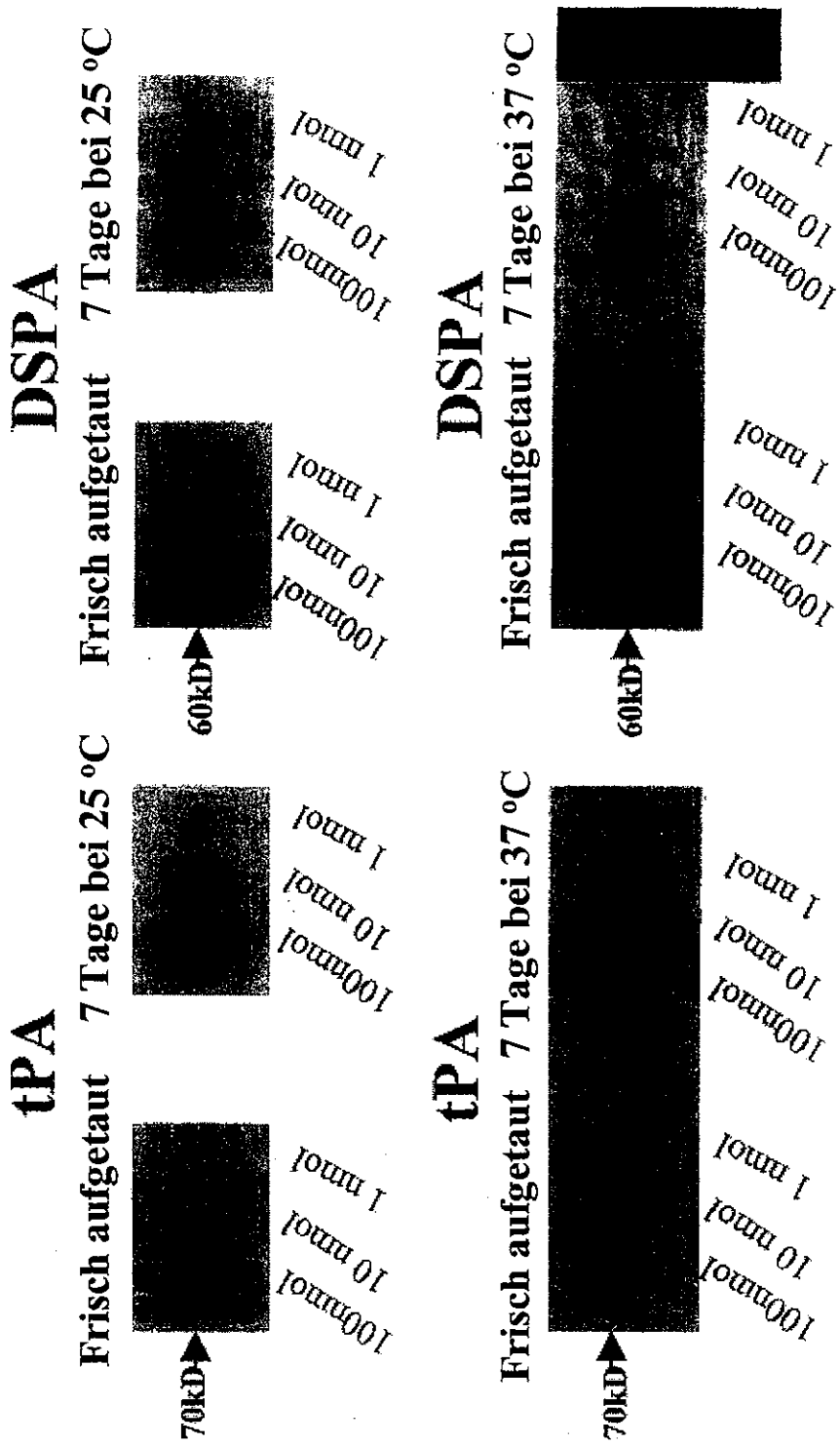
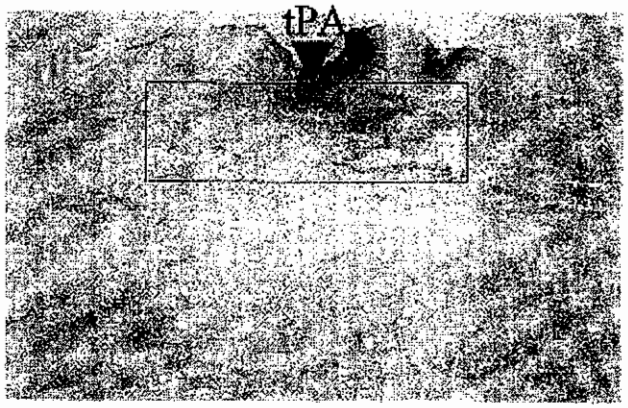

DSPA
Frisch aufgetaut 7 Tage bei 25 °C
1 mmol
10 mmol
100mmol
60kD
DSPA
Frisch aufgetaut 7 Tage bei 37 °C
1 mmol
10 mmol
100mmol
60kD
tPA
Frisch aufgetaut 7 Tage bei 25 °C
1 mmol
10 mmol
100mmol
70kD
tPA
Frisch aufgetaut 7 Tage bei 37 °C
1 mmol
10 mmol
100mmol
70kD

Fig. 1

Fig. 2

tPA: Immunhistochemie





tPA: Zymographie in situ



Nisselfärbung

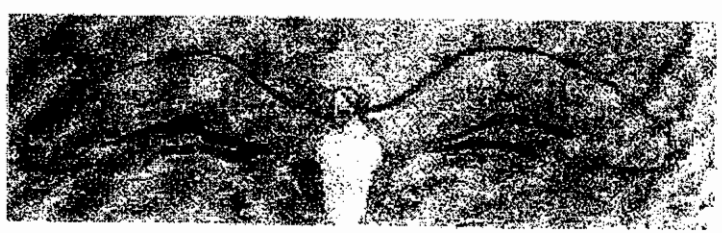


Fig. 4a

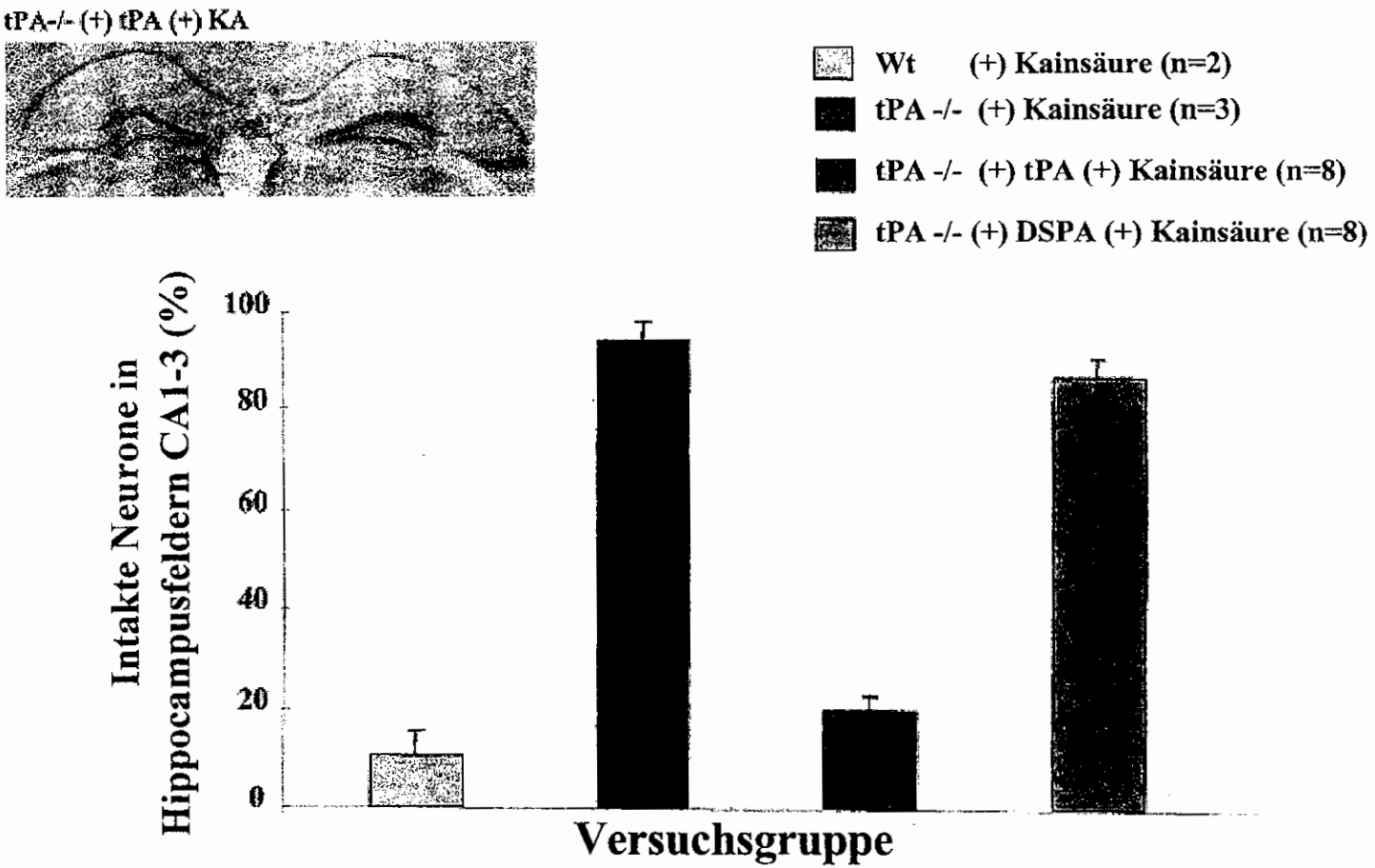

tPA-/- (+) tPA (+) KA
Wt (+) Kainsäure (n=2)
tPA -/- (+) Kainsäure (n=3)
tPA -/- (+) tPA (+) Kainsäure (n=8)
tPA -/- (+) DSPA (+) Kainsäure (n=8)
Intakte Neurone in Hippocampusfeldern CA1-3 (%)
100
80
60
40
20
0
Versuchsgruppe

Fig. 4b

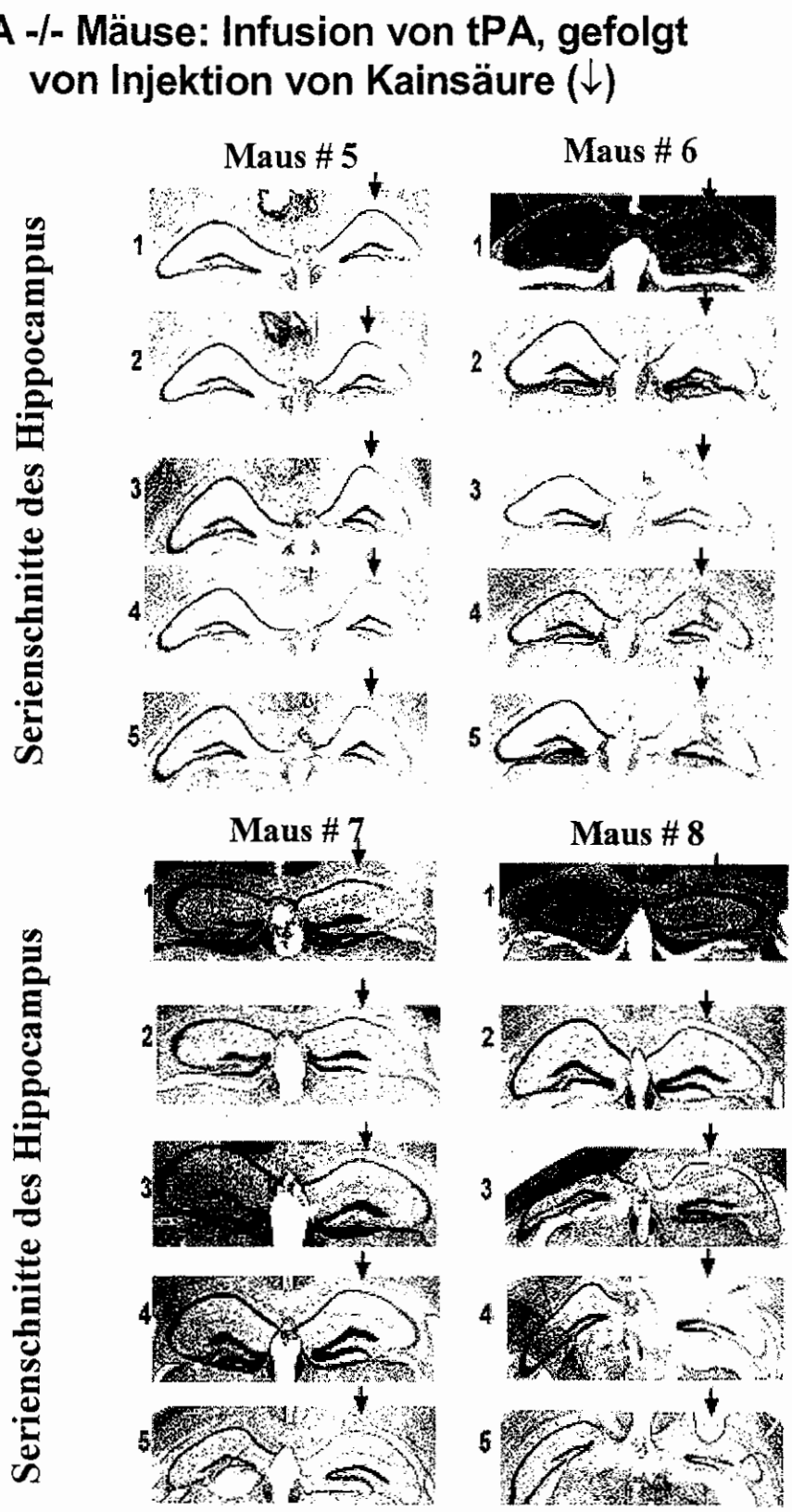
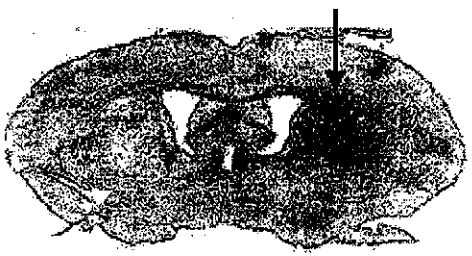
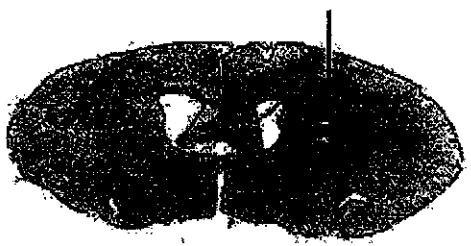

tPA -/- Mäuse: Infusion von tPA, gefolgt von Injektion von Kainsäure (↓)
Maus # 5
Maus # 6
Maus # 7
Maus # 8
Serienschnitte des Hippocampus
Serienschnitte des Hippocampus
1
2
3
4
5

Fig. 7

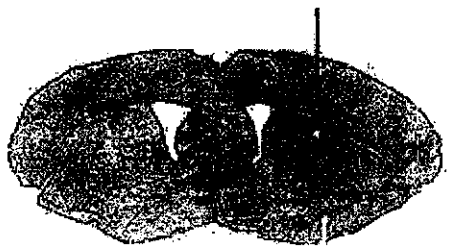
NMDA allein



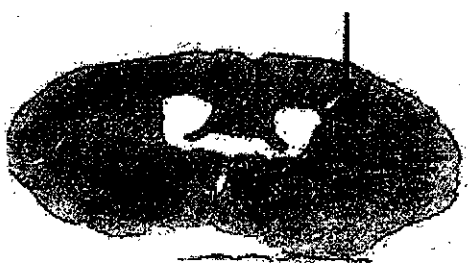
NMDA + t-PA



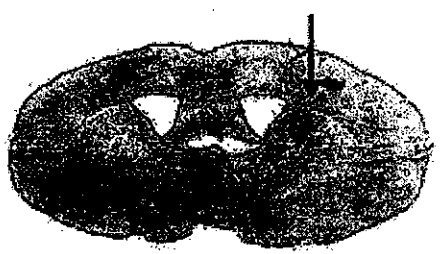
NMDA + DSPA



t-PA allein



DSPA allein



NMDA-Konzentration: 50 µM
tPA und DSPA: jeweils 46 µM
Injektionsort: s. Pfeil

Fig. 9

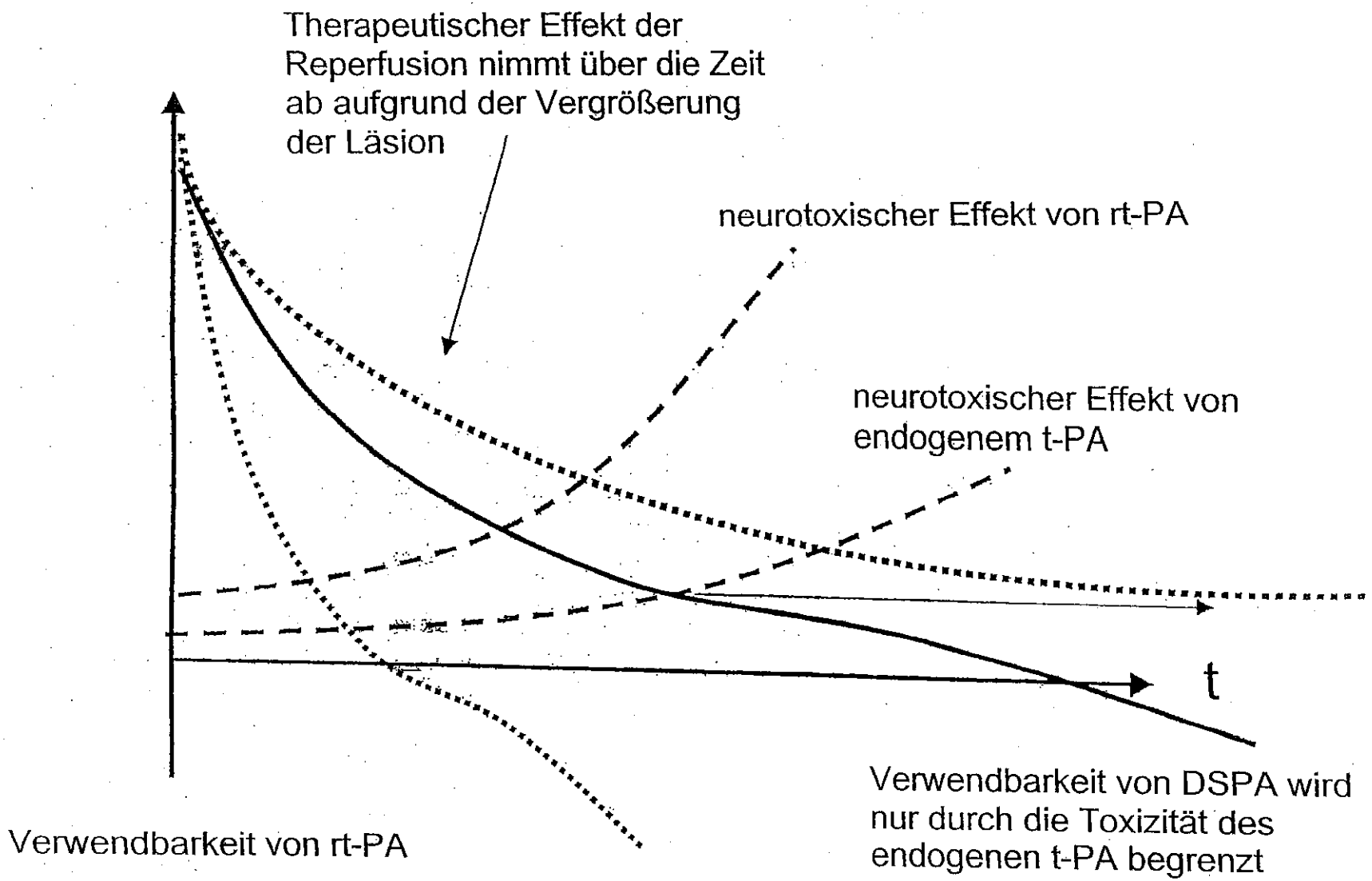
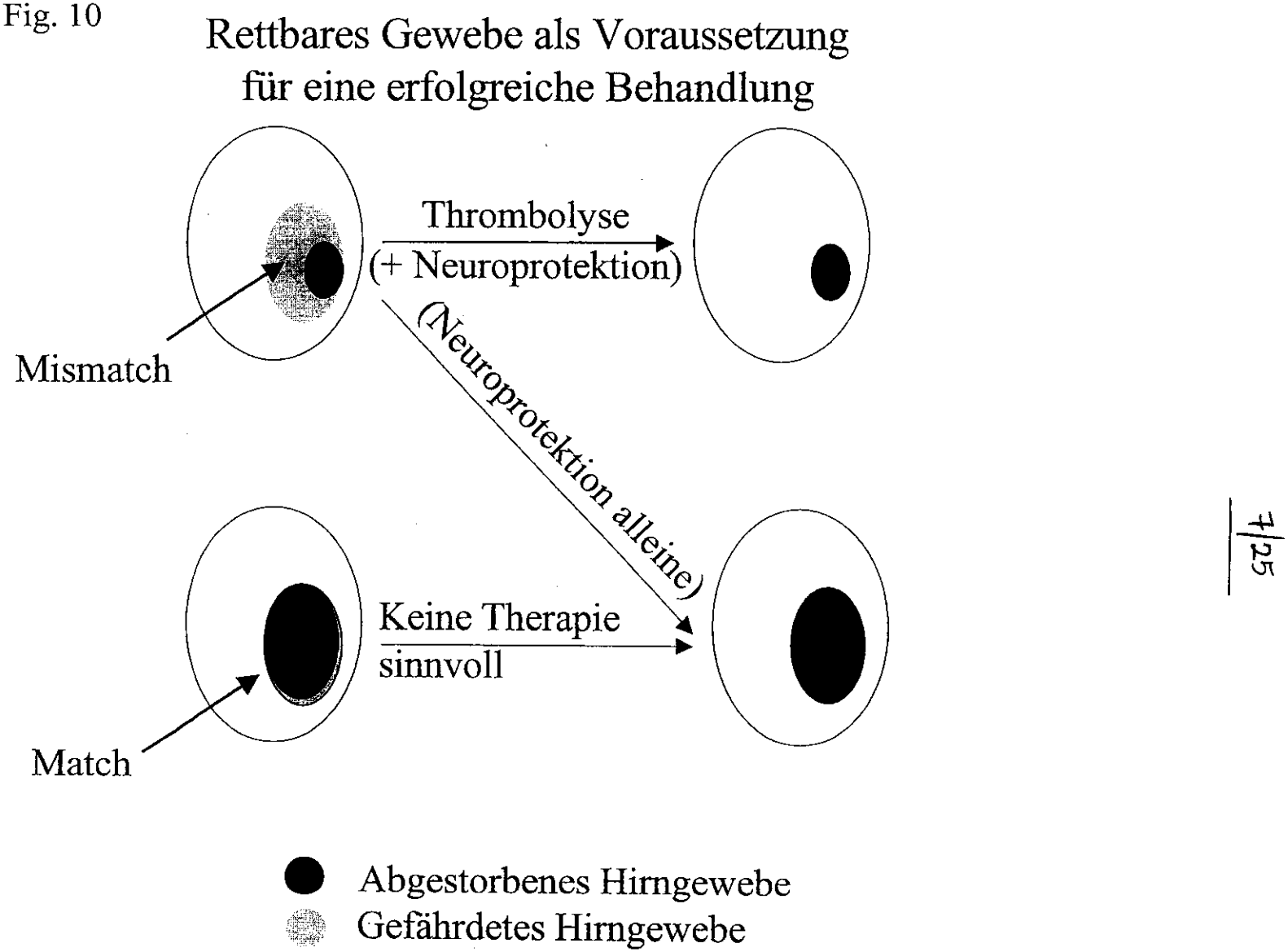

Therapeutischer Effekt der Reperfusion nimmt über die Zeit ab aufgrund der Vergrößerung der Läsion
neurotoxischer Effekt von rt-PA
neurotoxischer Effekt von endogenem t-PA
t
Verwendbarkeit von rt-PA
Verwendbarkeit von DSPA wird nur durch die Toxizität des endogenen t-PA begrenzt

Fig. 10


Rettbares Gewebe als Voraussetzung
für eine erfolgreiche Behandlung
Thrombolyse
(+ Neuroprotektion)
Mismatch
(Neuroprotektion alleine)
Keine Therapie
sinnvoll
Match
Abgestorbenes Hirngewebe
Gefährdetes Hirngewebe

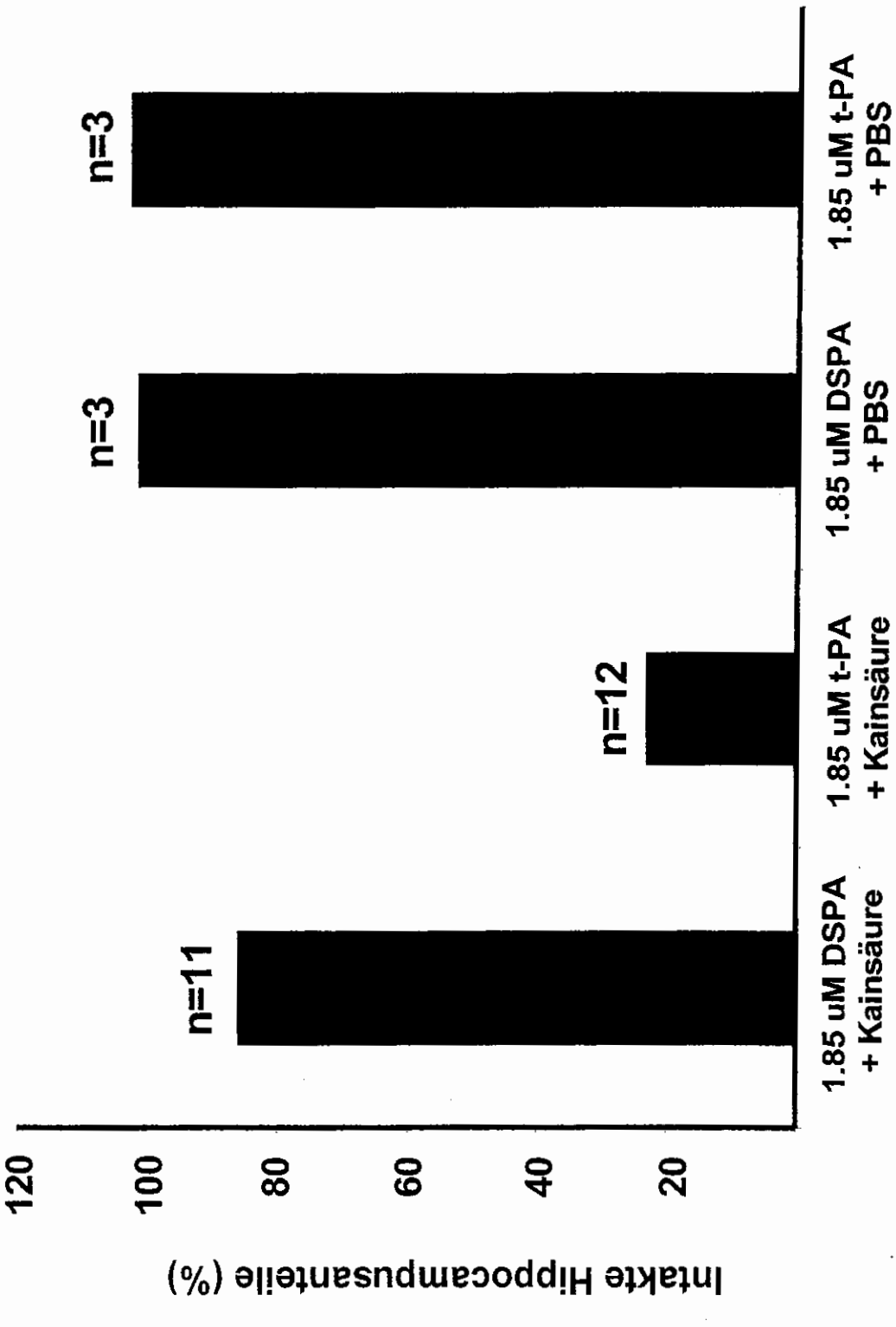

Intakte Hippocampusanteile (%)
120
100
80
60
40
20
n=11
n=12
n=3
n=3
1.85 uM DSPA + Kainsäure
1.85 uM t-PA + Kainsäure
1.85 uM DSPA + PBS
1.85 uM t-PA + PBS

Fig. 11

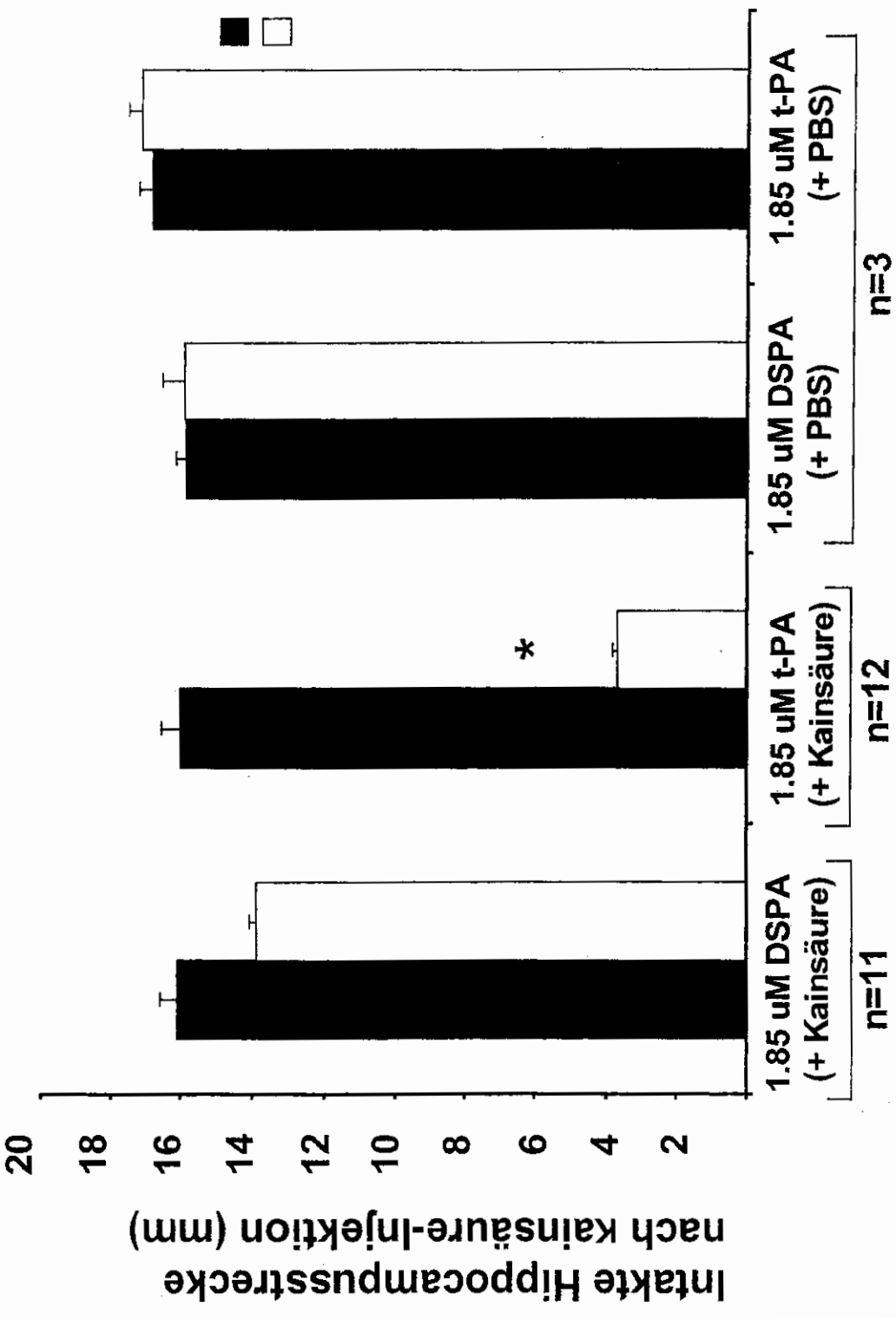

Intakte Hippocampusstrecke
nach kainsäure-Injektion (mm)
20
18
16
14
12
10
8
6
4
2
Kontralateral
Ipsilateral
* P<0.0001
*
1.85 uM DSPA
(+ Kainsäure)
n=11
1.85 uM t-PA
(+ Kainsäure)
n=12
1.85 uM DSPA
(+ PBS)
1.85 uM t-PA
(+ PBS)
n=3

Fig. 12

Fig. 13

SEQ. ID Nr. 2

```
  1  MVNTMKTKLL CVLLLCGAVF SLPRQETYRQ LARGSRAYGV ACKDEITQMT
 51  YRRQESWLRP EVRSKRVEHC QCDRGSNELH QVPSNSCDEP RCLNGGTCVS
101  NKYFSIHWCN CPKKFGGQHC EIDKSKTCYE GNGHFYRGKA STDTMGRPCL
151  PWNSATVLQQ TYHAHRSDAL QLGLGKHNYC RNPDNRRRPW CYVQVGLKPL
201  VQECMVHDCA DFQCGQKTLR EPRFHSTGGE FTTIENQPWF AAIYRRHRGG
251  SGVTYVCGGS LMSPCWVISA THCFIDYPKK EDYIVYLGRS RLNSNTQGEM
301  KFEVENLILH KDYSADTHHN DIALLKIRSK EGRCAQPSRT IQTICLPSMY
351  NDPQFGTSCE ITGFGKENST DYLYPEQLKM TVVKLISHRE CQQPHYYGSE
401  VTTKMLCAAD PQWKEIYPNV TDSCQGDSGG PLVCSLQGRM TLTGIVSWGR
451  GCALKDKPGV YTRVSHFLPW IRSHTKL
```

Fig. 14

SEQ. ID Nr. 1

```
  1  MVNTMKTKLL CVLLLCGAVF SLPRQETYRQ LARGSRAYGV ACKDEITQMT
 51  YRRQESWLRP EVRSKRVEHC QCDRGQARCH TVPVKSCSEP RCFNGGTCQQ
101  ALYFSDFVCQ CPEGFAGKCC EIDTRATCYE DQGISYRGTW STAESGAECT
151  NWNSSALAQK PYSGRRPDAI RLGLGNHNYC RNPDRDSKPW CYVFKAGKYS
201  SEFCSTPACS STCGLRQYSQ PQFHSTGGLF ADIASHPWQA AIFAKHRRSP
251  GERFLCGGIL ISSCWILSAA HCFQERFPPH HLTVILGRTY RVVPGEEEQK
301  FEVEKYIVHK EFDDDTYDND IALLQLKSDS SRCAQESSVV RTVCLPPADL
351  QLPDWTECEL SGYGKHEALS PFYSERLKEA HVRLYPSSRC TSQHLLNRTV
401  TDNMLCAGDT RSGGPQANLH DACQGDSGGP LVCLNDGRMT LVGIISWGLG
451  CGQKDVPGVY TKVTNYLDWI RDNMRP
```

Fig. 15 (Tab. 1)

		Hippocampal length intact (mm)		
Treatment group	**Number of animals**	**Contralateral side** mean (SEM)	**Ipsilateral side** mean (SEM)	**Percentage remaining**
t-PA infusion (1.85 uM) + KA	12	15.99 (0.208)	3.63 (0.458)	22.7*
DSPA infusion (1.85 uM) + KA	11	16.07 (0.124)	13.8 (0.579)	85.87
t-PA infusion (1.85 uM) + PBS	3	16.75 (0.381)	17.08 (0.363)	101.97
DSPA infusion (1.85 uM) + PBS	3	15.75 (0.629)	15.83 (0.363)	100.50
t-PA infusion (0.185 uM) + KA	3	15.60 (0.702)	5.07 (1.09)	32.5
DSPA infusion (18.5 uM) + KA	3	16.06 (0.176)	13.80 (1.22)	85.93

* $P < 0.0001$

Fig. 16 (Tab. 2)

Treatment group	Number of wild-type mice	Mean lesion volume (mm^3) (SEM)
NMDA alone	8	1.85 (0.246)
NMDA + t-PA	8	3.987 (0.293) *
NMDA + DSPA	8	1.656 (0.094) **
t-PA alone	3	0.20 (0.011)
DSPA alone	3	0.185 (0.016)

* $P < 0.0001$

** Not significant

Fig. 17 (Tab. 3)

Patient		NIHSS				
	Baseline	Post Tmt	Day 7	Day 30	Day 90	sAEs
1001	12	7	4	4	*	Re-Infarction
1002	8	9	2	0	0	
1003	8	10	12	10	*	
1004	8	4	2	0	0	
1005	11	11	4	5	*	
1006	9	7	1	*	*	
1007	14	6	*	*	*	
2001	19	20	--	--	--	ICH, death
2002	15	21	--	--	--	ICH, death
3001	8	7	6	5	*	
3002	15	16	9	8	*	
3003	10	19	21	*	--	Death day 39

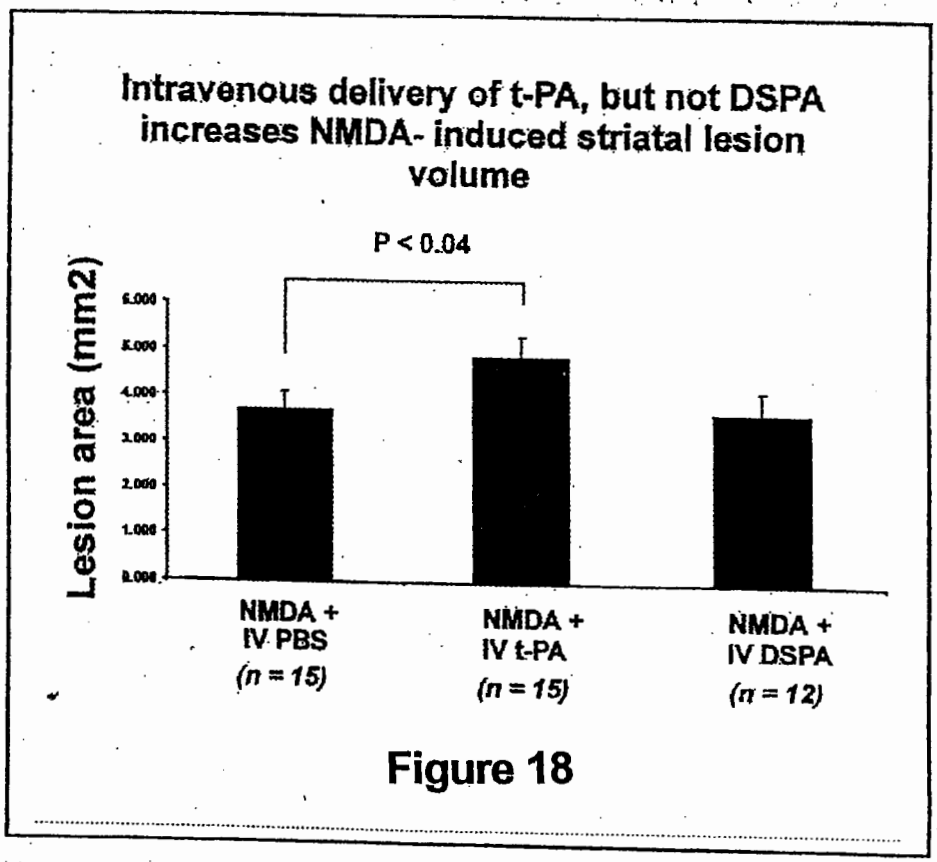
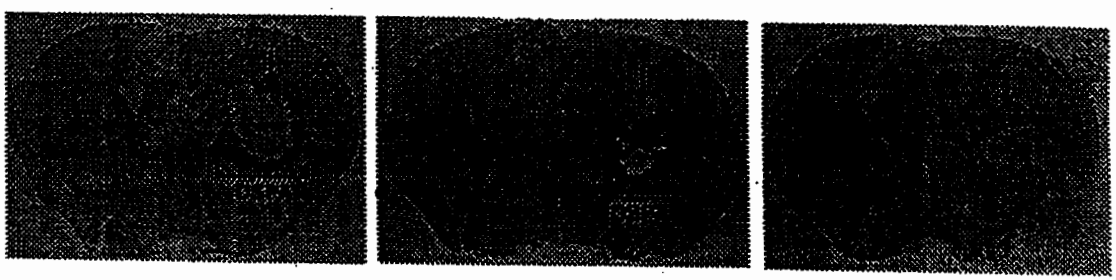

Intravenous delivery of t-PA, but not DSPA increases NMDA- induced striatal lesion volume
P < 0.04
Lesion area (mm2)
NMDA + IV PBS (n = 15)
NMDA + IV t-PA (n = 15)
NMDA + IV DSPA (n = 12)

Figure 18

DSPA injected intravenously after NMDA-induced injury crosses the blood-brain-barrier



Immunohistochemistry for DSPA in coronal sections of wild-type mice
Panel A: Mouse stereotaxically injected into the striatal region with NMDA. 24 hour later DSPA injected into the tail vein. Sections prepared after a further 24 hour.
Panel B: Mouse stereotaxically injected with NMDA+DSPA into the striatal region. Sections prepared 24 h later
Panel C: Stereotaxic injection of NMDA alone. Section prepared after 24 hours.
Arrows indicate NMDA injection site.

Figure 19

Below are the data up to day 7 of the first 47 patients (unblinded), the data of all patients of the 62.5µg dose tier (blinded and completed with 19 patients; 15 Desmoteplase and 4 placebo) and the patients of the 90µg (blinded and ongoing, 15 patients recruited (3 or 4 on placebo and 10 or 11 on Desmoteplase – distribution of numbers based on the blocks used for randomisation; acute data complete, data at day 7 available of 13 patients).

Dose allocation

Patient Population

DIAS: unblinded

- **16 Placebo patients**
- **17 patients treated with 25mg (312.5µg/kg median)**
- **13 patients treated with 37.5mg or 50mg (545.5µg/kg median)**
  (7 with 37.5mg, 6 with 50mg)

DIAS and DEDAS: still blinded

- **19 Patients (4 Placebo, 15 treated with 62.5µg/kg) (DIAS)**
- **17 Patients (≥3 placebo, ≥ 12 treated with 90µg/kg) (15 DIAS, 2 DEDAS)**

Fig. 20

Figure 21(below) describes the most relevant baseline data comparing all recruited patients so far. It is important to note that the 90µg group seems to have more patients with a higher age (risk factor for bleeding) and that there seems to be a trend for later time to treatment. This is however true for comparing the "old" and "new" patients and is probably due to the fact the treatment window was changed during the first part of the DIAS trial. One symptomatic bleeding occurred in 90µg dose group so far and none in the 62.5µg tier. The NIHSS (stroke severity) at entry is comparable to the 25mg group. None of the differences between the groups are thought to have any statistical or medical relevance, since there seems to be a good overlap and spread amongst the groups.

Note that the graphs below represent the upper and lower quartiles. The median is outlined as a prominent line. Blue graphs represent data from the interim analysis, red represent the 62.5µg tier and green the 90µg tier.

: Baseline characteristics

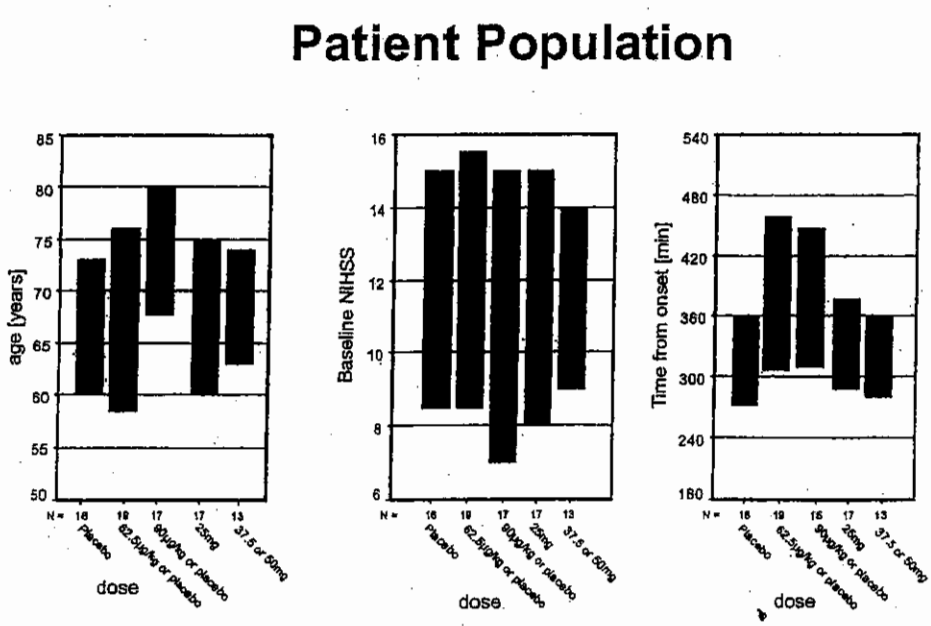


Fig. 21

Figure 22 (below) describes the key issue of the safety aspects of the study (sICH). There has been one symptomatic bleeding in the 36 patients (62.5 and 90µg/kg dose tiers) since the study has been restarted. In comparison to the previous bleedings several aspects are of interest. The bleeding occurred rather late (28h after treatment), this seems to be the first patient that did not have any of the previous present risk factors (age, high glucose level, high NIHSS). The asymptomatic transformations seem to show no dose dependency, which is in line with a substantial fairly high incidence in untreated patients as is known from previous published trials. The patient who had sICH showed signs of reperfusion and at the time of 4-8h MRI also signs of asymptomatic hemorrhagic transformation.

Asymptomatic and Symptomatic Hemorrhagic Transformations

Safety: ICHs and deaths

		dose					
		Placebo	25mg	37.5 or 50mg	62.5µg/kg or placebo	90µg/kg or placebo	
hemorrhagic transformation	no	14	11	7	13	14	59
		87,5%	64,7%	53,8%	68,4%	82,4%	72,0%
	asymptomatic	2	2	2	6	2	14
		12,5%	11,8%	15,4%	31,6%	11,8%	17,1%
	symptomatic	0	4	4	0	1	9
			23,5%	30,8%		5,9%	11,0%
		16	17	13	19	17	82
		100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

		dose					
		Placebo	25mg	37.5 or 50mg	62.5µg/kg or placebo	90µg/kg or placebo	
mortality	dead	0	2	5	1	2	10
			11,8%	38,5%	5,3%	11,8%	12,2%
	alive	16	15	8	18	15	72
		100,0%	88,2%	61,5%	94,7%	88,2%	87,8%
		16	17	13	19	17	82
		100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

Fig. 22

Figure 23 describes vessel opening as measured by TIMI change. As has been stated by some of the investigators at the recent investigator meetings that MRA underrates the vessel opening. We will need to address this in the evaluation with the new TISS scale (this scale has been designed for the DIAS and DEDAS trial, representing a combination of TIMI and PWI changes, which has been adapted based on the data of the first 47 patients and is used in an exploratively manner). Reperfusion as assessed by PWI (Figure 25 gives a slightly different picture. One has to keep in mind that the TIMI scale shows the reperfusion of larger vessels only, whereas PWI is a consecutive (i.e. later) change of blood flow into the penumbra, which also can reflect an improvement of the microcirculation. Thus it seems logical that the timelines and the "kinetics"/magnitude of these measures appear to follow slightly different algorithms. However one would have to be alarmed if they would show into different directions. In both modes the 90µg group reaches values, which are similar to the 25mg group, which was the best group of the "old" patients (i.e. the first unblinded data set of the interim analysis) . It is difficult to asses if the "real" opening rate is eventually higher or a bit lower, but it is definitively better than the placebo group. For raw data see Tables 1 and 2 below.

TIMI change

Efficacy: „TIMI" change

Comparing „old" and „new"

		dose					
		Placebo	25mg	37.5 or 50mg	62.5µg/kg or placebo*	90µg/kg or placebo*	
TIMI change	no improvement	11	8	6	14	10	49
		68,8%	57,1%	50,0%	77,8%	71,4%	66,2%
	re-opening (>=1)	5	6	6	4	4	25
		31,3%	42,9%	50,0%	22,2%	28,6%	33,8%
		16	14	12	18	14	74
		100,0%	100,0%	100,0%	100,0%	100,0%	100,0%

* judged by center (not quantitatively)

available data of central analysis N= 10 for the 90µg tier

dose	
62.5µg/kg or placebo	90µg/kg or placebo
11	7
84,6%	70,0%
2	3
15,4%	30,0%
13	10
100,0%	100,0%

Fig. 23

Reperfusion as indicated by reduction on PWI

Efficacy: Relative PWI change*

Comparing „old“ and „new“

Rate of Responders (all patients)

		dose					
		Placebo	25mg	37.5 or 50mg	62.5µg/kg or placebo*	90µg/kg or placebo*	
perfusion deficit volume 4-8h	< 30% smaller or larger than baseline	12 80,0%	7 46,7%	5 55,6%	12 92,3%	9 60,0%	45 67,2%
	>=30% smaller than baseline	3 20,0%	8 53,3%	4 44,4%	1 7,7%	6 40,0%	22 32,8%
		15 100,0%	15 100,0%	9 100,0%	13 100,0%	15 100,0%	67 100,0%

* judged by center: "smaller" vs. "not smaller" than baseline

available data of central analysis N= 10 for the 90µg tier

dose	
62.5µg/kg or placebo	90µg/kg or placebo
11 91,7%	3 30,0%
1 8,3%	7 70,0%
12 100,0%	10 100,0%

Fig. 24

TIMI, PWI and TISS at 4-8h

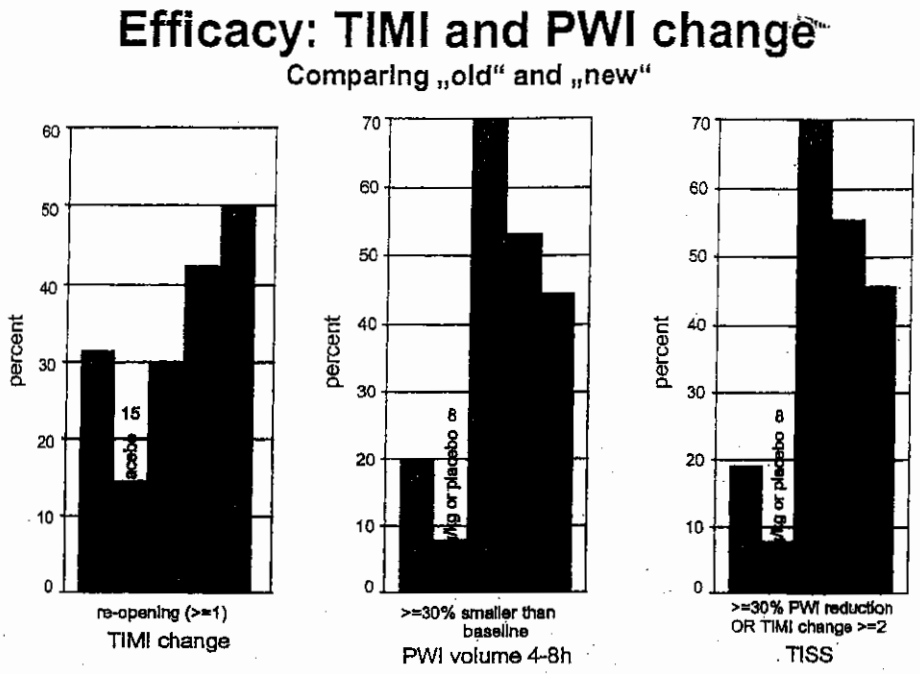


Figure 25 shows a sign of a bell shaped dose response curve for PWI but not for TIMI. Whereas a dose increase for TIMI seems to be there when the TIMI and TISS already show a bell shaped curve may be explained by the fact that TIMI just looks at (larger) vessels, while PWI and TISS look at reperfusion effects on tissue. Whereas higher doses lead to more rapid and complete vessel opening this may lead also to some reperfusion damage which could be the reason for the bell shaped curve of the PWI and TISS results.

Fig. 25

Figure 26 (below) shows how the reperfusion translates into the acute clinical improvement of the stroke patients measured as improvement of NIHSS stroke scale. It is amazing that both the 62.5µg/kg and the 90µg do better than any dose group at 4-8h after medication. One might speculate that this is an indicator that reperfusion injury occurred in the previous higher dosages. This trend is continued up to day 7, where both dose groups do better than the best of the previous groups (25mg) both when bleedings are included or excluded in the analysis. An important point is that the 62.5µg/kg dose group is as good as the 25mg and the 90µg/kg is better than the 25mg at day 7.

NIHSS improvement 4-8h after treatment and day 7

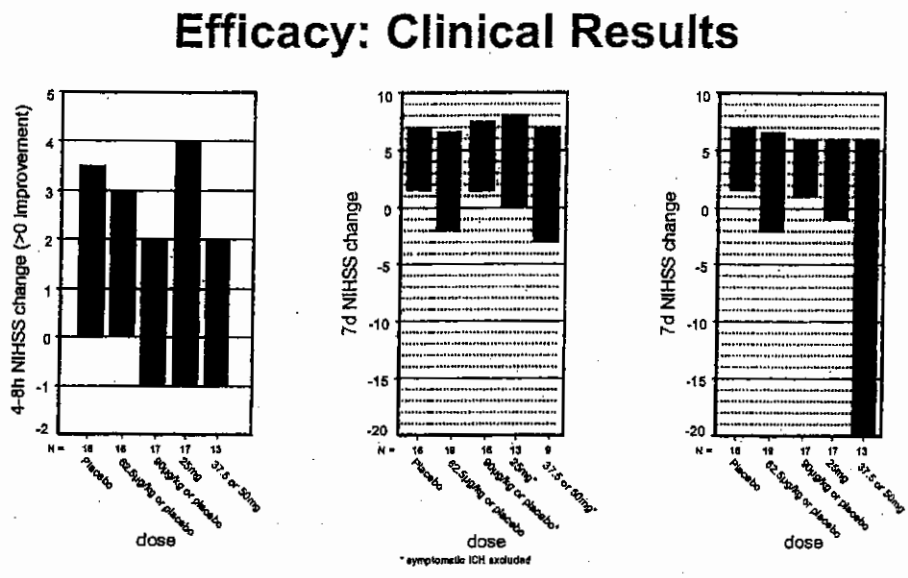


Fig. 26

Figure 27 Hypothetical Dose response curve for Desmoteplase in stroke patients

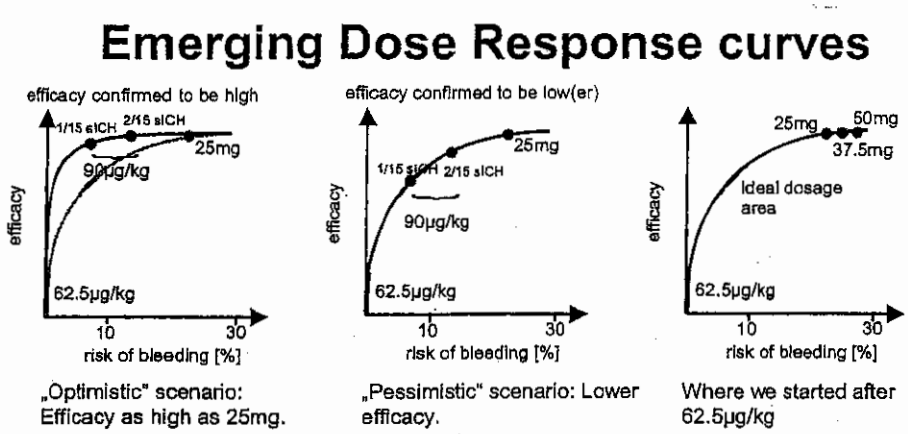


Fig. 27

Below are the raw blinded data of the 62.5µg/kg (Table4) and the 90µg/kg (Table 5) dose tier

Table 4: key raw data of the 19 patients in the 62.5µg dose group

Date	Pat. No.	Baseline data				4-8 hours outcome			72 hours outcome	Day 7 NIHSS
		NIHSS	Age	Weight	Onset	ICH	Reperf.	NIHSS		
26.08.02	601	9	44	72	5h 10m	No	No	6	no bleed no SAE	1
20.09.02	602	8	78	80	8h 04m	No	Yes	14	no bleed no SAE	12
21.09.02	603	18	42	72	6h 50m	No	No	17	no bleed no SAE	11
26.09.02	604	9	79	75	8h 15m	No	No significant changes	8	no bleed no SAE	11
30.09.02	605	13	71	90	4h 54m	No	No	13	no bleed no SAE	16
16.10.02	606	18	73	90	5h 25m	No	No	19	no bleed no SAE	15
17.10.02	607	18	76	65	4h 50m	No	No	25	no bleed no SAE	19
30.10.02	608	8	67	91	9h 00m	No	No	5	no bleed no SAE	6
01.11.02	609	15	70	90	7h 25m	No	MRI not done (only FLAIR), intubation 5,5h after Rx	15	no bleed mech. Vent.	17
07.11.02	610	15	81	74	6h 00m	No	No	8	no bleed no SAE	8
13.11.02	611	7	46	119	4h 15m	No	No significant changes	7	no bleed no SAE	3
21.11.02	612	14	71	52	8h 10m	No	Yes. Partial reperf.	12	asympt.bl.	2
21.11.02	613	9	50	52	5h 30m	No	Yes	8	asympt.bl.	9
03.12.02	614	16	65	67	5h 40m	asym.	Yes. Partial reperf.	13	no SAE	10
08.12.02	615	8	64	68	8h 30m	No	No	8	no bleed no SAE	1
11.12.02	616	14	76	61	5h 00m	No	No	18	no bleed no SAE	27
17.12.02	617	8	53	120	4h 15m	No	No	5	no bleed no SAE	14
19.12.02	618	11	82	85	6h 00m	asym	No	11	no SAE	8
22.12.02	619	16	70	100	7h 00m	No	No	16	no bleed no SAE	15

Fig. 28 (Tab. 4)

Table 5: key raw data of the first 17 patients (15 DIAS and 2 DEDAS patients) in the 90µg dose group

Date	Pat. No.	Centre	Baseline data				4-8 hours outcome			72 hours outcome	Day 7 NIHSS
			NIHSS	Age	Weight	Onset	ICH	Reperf.	NIHSS		
04.01.03	701	Chang	15	46	53	8h 30m	No	No	22	no bleed no SAE	14
27.01.03	702	Samson	19	81	101	8h 52m	No	No	21	no bleed no SAE	7
29.01.03	703	Hacke	14	51	60	5h 30m	No	Yes. Smaller PWI	8	no bleed no SAE	5
04.02.03	704	Rouanet	6	32	53	6h 00m	No	No	4	no bleed no SAE	2
05.02.03	705	Davalos	7	69	70	8h 10m	No	No	6	asympt.bl. no SAE	2
05.02.03	706	Davalos	6	80	65	5h 00m	No	No	6	no bleed no SAE	4
07.02.03	707	Chabriat	16	80	52	3h 50m	No	Yes. Reopening on MRA	14	no bleed no SAE	11
08.02.03	708	Fazekas	11	81	90	7h 00m	Asym	*Yes. Smaller PWI and reopening on MRA*	9	no SAE	2
12.02.03	709	Blecic	12	77	71	4h 30m	No	Some regression of PWI	12	no bleed no SAE	12
19.02.03	710	Levi	9	85	70	5h 00m	No	Yes. Partial reopening on MRA and partial reperfusion on PWI	9	no bleed no SAE	3
25.02.03	711	Davalos	12	43	50	8h 23m	No	No	13	no bleed no SAE	6
28.02.03	712	Bogouss	17	68	70	5h 15m	No	No	17	no bleed no SAE	12
04.03.03	713	Meves	14	78	80	5h 10m	No	Patient died. Pulm. Embolism?	-	-	-
10.03.03	714	Davalos	6	69	78	6h 10m	Asym	Yes. Partial reopening on MRA and smaller PWI	4	Sympt. Bleed	19
12.03.03	715	Chabriat	6	75	78	7h 30m	No	Yes. Smaller PWI	4	no bleed no SAE	2
27.03.03	716	Chang	18	54	69	6h 40m	Asym	Yes. Minimal reperf. on MRA.	19	no SAE	
29.03.03	717	Hacke	11	52	80	5h 25m	No	No	11		
30.03.03	718	Ludolph	5	59	58	6h 45m	No	Yes. Reopening on MRA and smaller PWI.	4		
22.02.03	201		19	72	99	Not treated	---	—	—	—	—
03.03.03	202		12	70	92	7h 20m	No	No Patient showed signs of progressive basilary artery occlusion	34	Patient on mech vent.	Patient died on day 6 (1)
19.03.03	203		16	77	57	9h 00m	No	Yes. Partial reopening on MRA	16	no bleed no SAE	

Fig. 29 (Tab. 5)