All expts. carried out in adult MPS I (IDUA deficient) mice

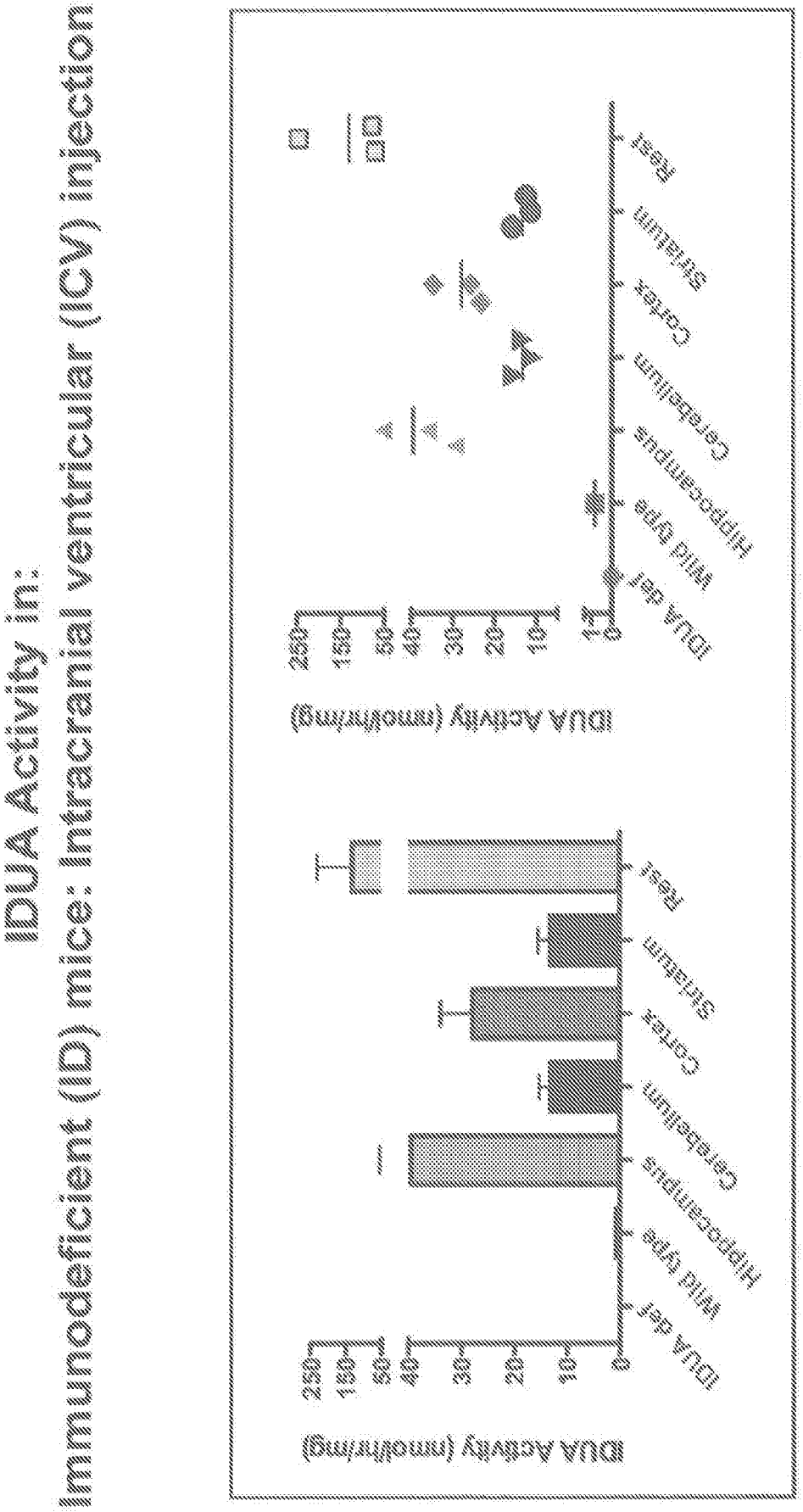
Route of Adm.	Mice	CP	Age at inj.	Sacrificed
1. ICV	Immunodeficient	No	4.5 mths	10 wks post inj.
2. ICV	Immunocompetent	Yes	4.5 mths	8 wks post inj.
3. Intrathecal	Immunocompetent	Yes	4.5 mths	11 wks post inj.
4. ICV	Immunotolerized	No	3 mths	9 wks post inj.

All animals injected with 10 ul AAV9 vector (3×10^{11} particles)

ICV injections: Right lateral ventricle
IT injections: Lumbar area

Fig 1

Fig 2


IDUA Activity in:
Immunodeficient (ID) mice: Intracranial ventricular (ICV) injection
IDUA Activity (nmol/hr/mg)
250
150
50
40
30
20
10
0
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest
IDUA Activity (nmol/hr/mg)
250
150
50
40
30
20
10
0
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest

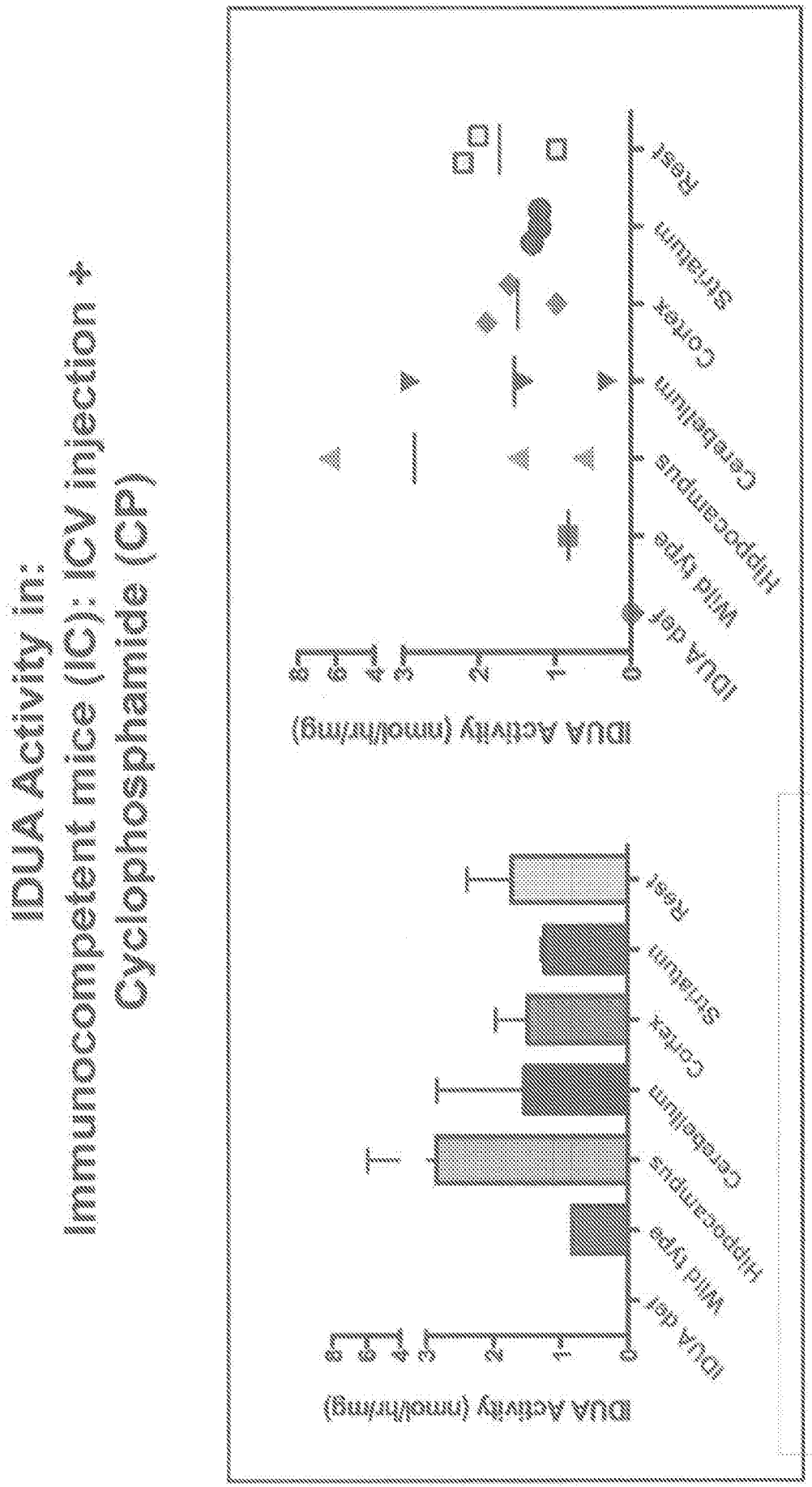
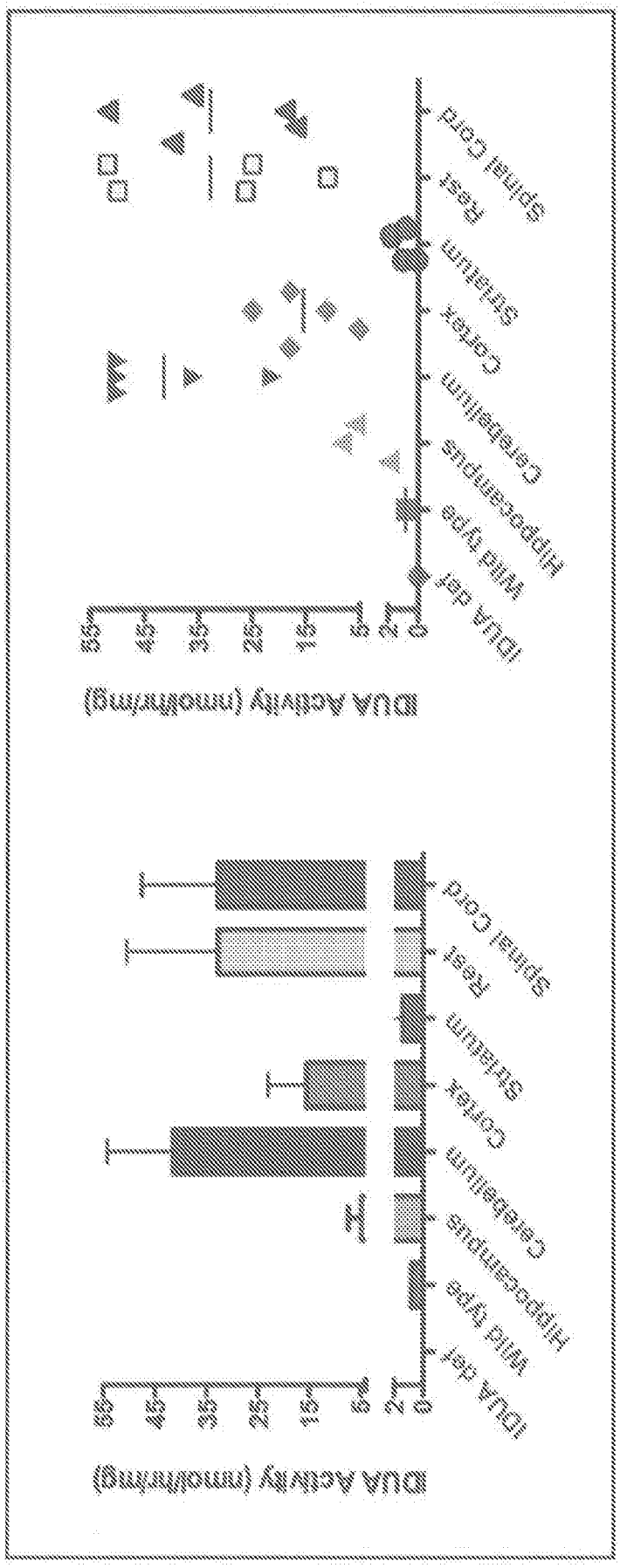

IDUA Activity in:
Immunocompetent mice (IC): ICV injection +
Cyclophosphamide (CP)
IDUA Activity (nmol/hr/mg)
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest
IDUA Activity (nmol/hr/mg)
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest

Fig 3

Fig 4A


IDUA Activity in:
Immunocompetent (IC) mice: Intrathecal (IT) injection
+ Cyclophosphamide (CP)
IDUA Activity (nmol/hr/mg)
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest
Spinal Cord
IDUA Activity (nmol/hr/mg)
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest
Spinal Cord

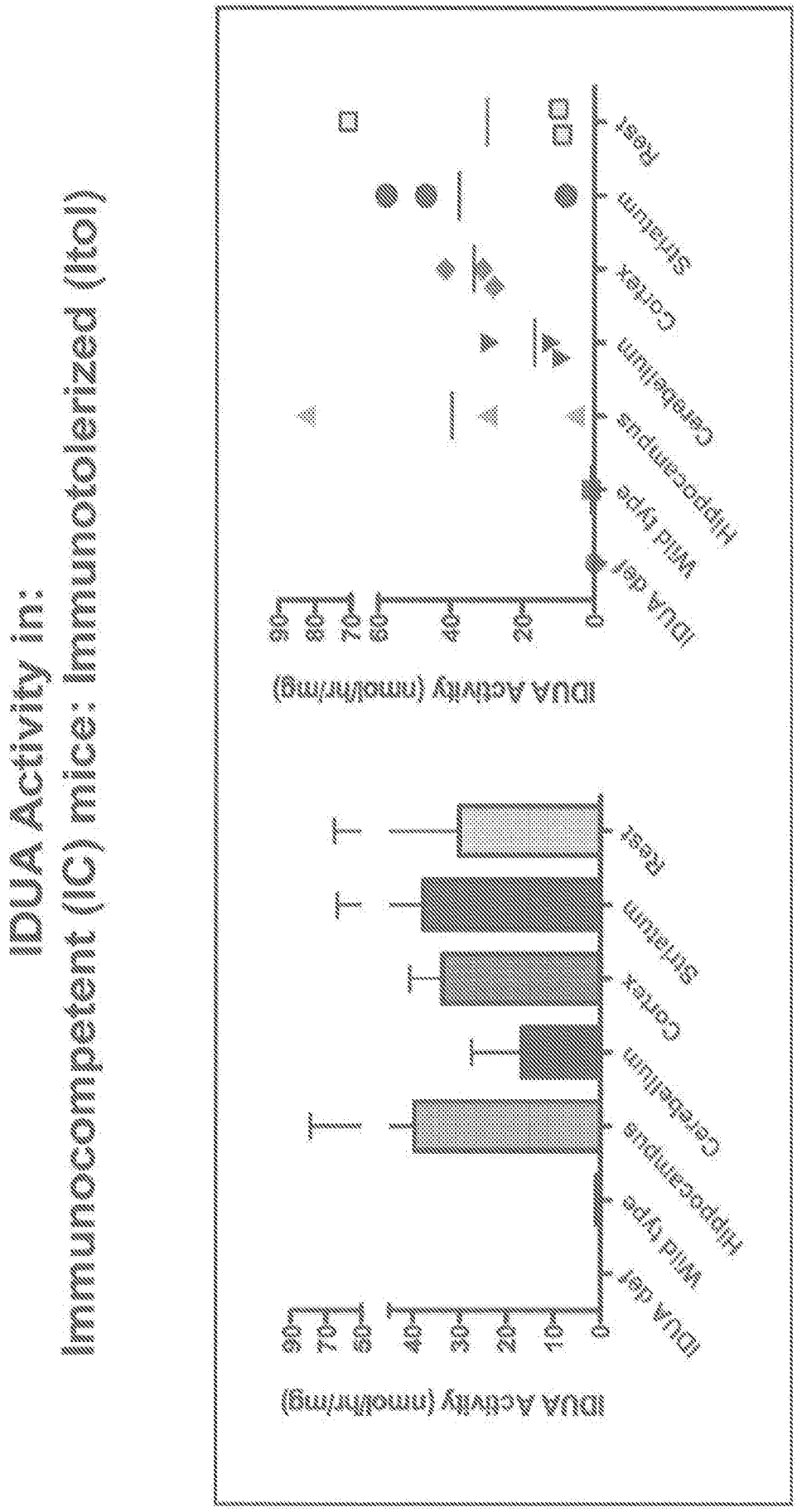
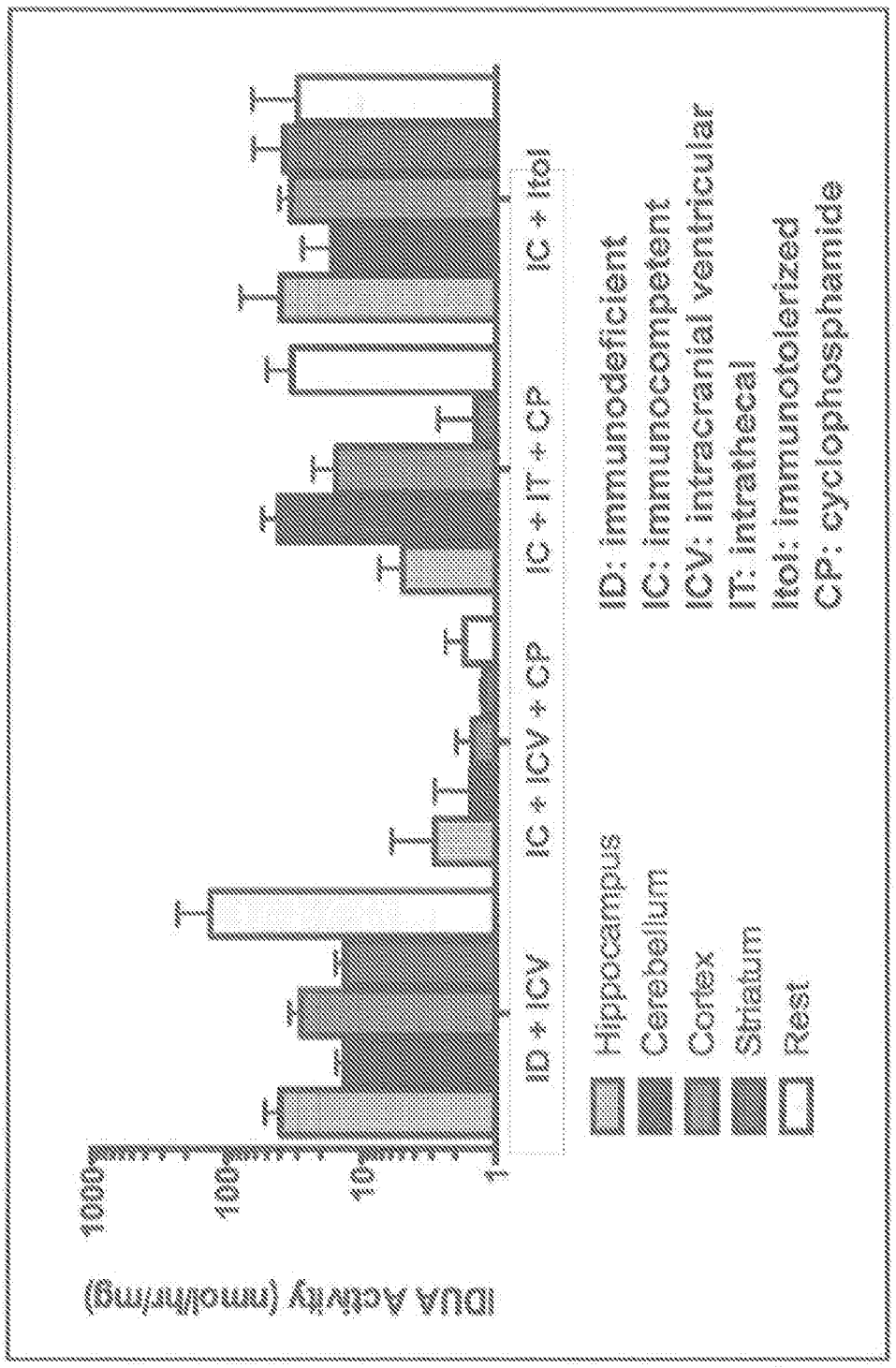

IDUA Activity in:
Immunocompetent (IC) mice: Immunotolerized (Itol)
IDUA Activity (nmol/hr/mg)
0
20
40
60
80
100
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest
IDUA Activity (nmol/hr/mg)
0
10
20
30
40
60
70
IDUA def
Wild type
Hippocampus
Cerebellum
Cortex
Striatum
Rest

FIG 5

Fig 6


Comparison of Different Routes of Delivery
IDUA Activity (nmol/hr/mg)
1000
100
10
1
ID + ICV
IC + ICV + CP
IC + IT + CP
IC + Itol
Hippocampus
Cerebellum
Cortex
Striatum
Rest
ID: immunodeficient
IC: immunocompetent
ICV: intracranial ventricular
IT: intrathecal
Itol: immunotolerized
CP: cyclophosphamide

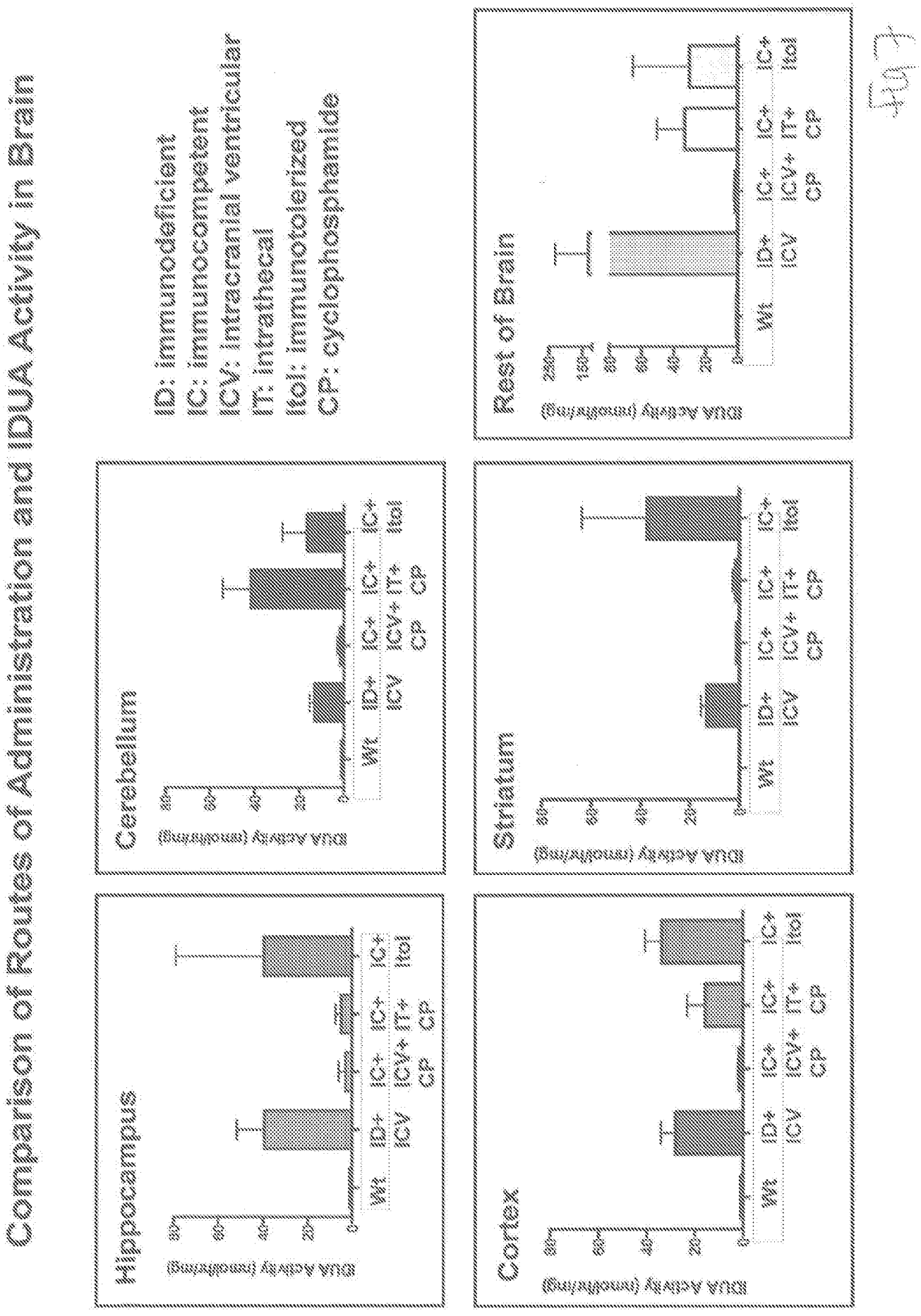

Comparison of Routes of Administration and IDUA Activity in Brain
ID: immunodeficient
IC: immunocompetent
ICV: intracranial ventricular
IT: intrathecal
Itol: immunotolerized
CP: cyclophosphamide
Hippocampus
Cerebellum
Striatum
Cortex
Rest of Brain
IDUA Activity (nmol/hr/mg)
Wt
ID+ ICV
IC+ ICV+ CP
IC+ IT+ CP
IC+ Itol

Fig. 7

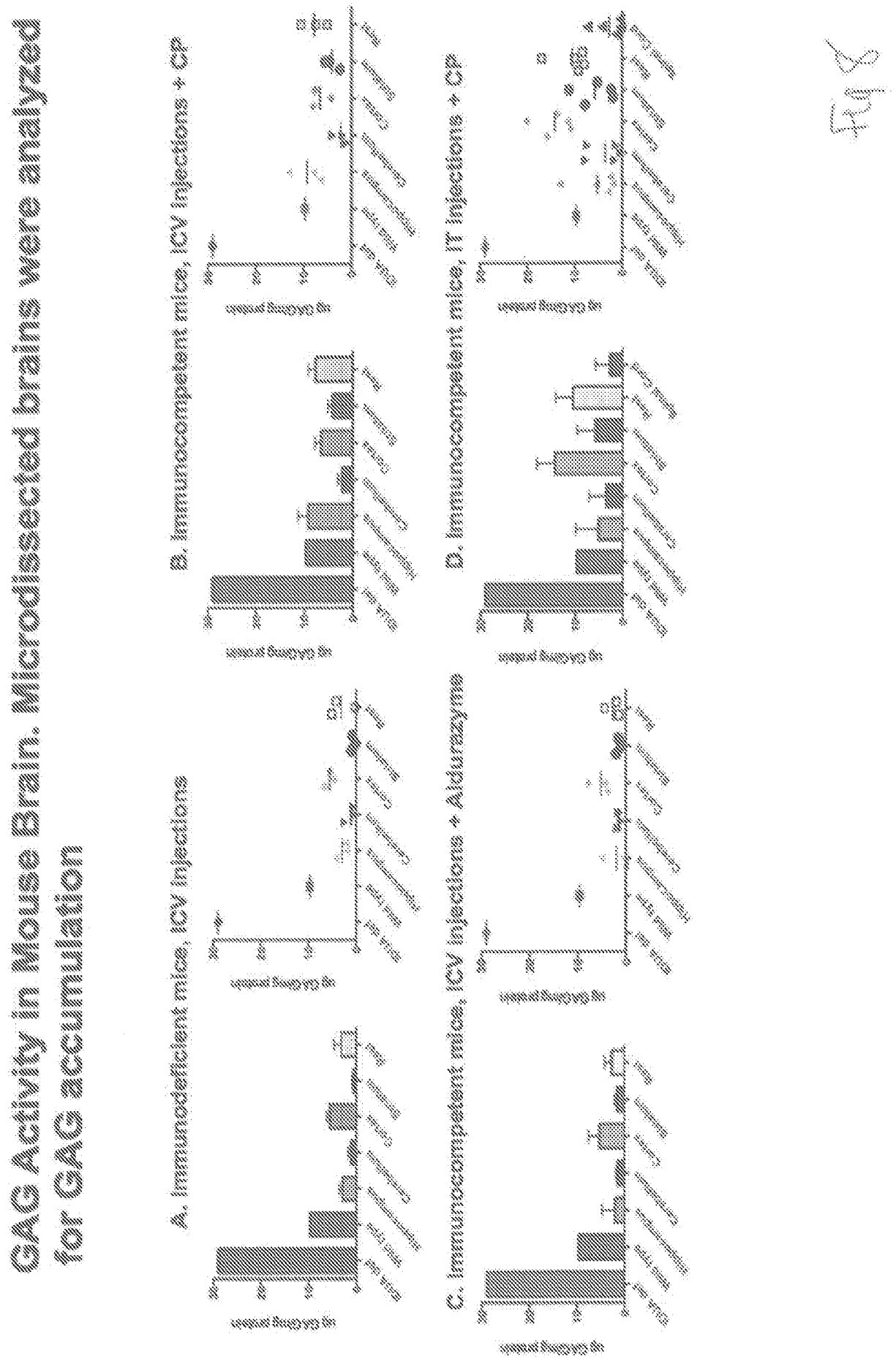

GAG Activity in Mouse Brain. Microdissected brains were analyzed for GAG accumulation
A. Immunodeficient mice, ICV injections
B. Immunocompetent mice, ICV injections + CP
C. Immunocompetent mice, ICV injections + Aldurazyme
D. Immunocompetent mice, IT injections + CP

Fig 4

Figure 9. Experimental design

Route of Vector administration	Immune competency	Immune modulation	Age of animals at injection	Expt. end point (post-injection)
ICV	Immunodeficient	None		10 weeks
ICV	Immunocompetent	Immunosuppression w/CP (partial regimen)	4.5 months	
			4.5 months	8 weeks
ICV	Immunocompetent	Immunotolerization	3 months	11 weeks
ICV	Immunocompetent	None		
ICV	Immunocompetent	Immunosuppression w/CP (full regimen)	3 months	
IT	Immunocompetent	Immunosuppression w/CP (full regimen)	3 months	9 weeks
		Enzyme tolerization	4.5 months	10 weeks
IT	Immunocompetent	None	4.5 months	11 weeks
IT	Immunocompetent			

Abbreviations:
ICV: Intracerebroventricular
IT: Intrathecal
CP: Cyclophosphamide

Expts. were carried out in adult MPS I (IDUA deficient) mice
All animals were injected with 10 µL AAV9 vector (3×10^{11} particles)

Figure 10. Intracranial Delivery into Immunodeficient Animals

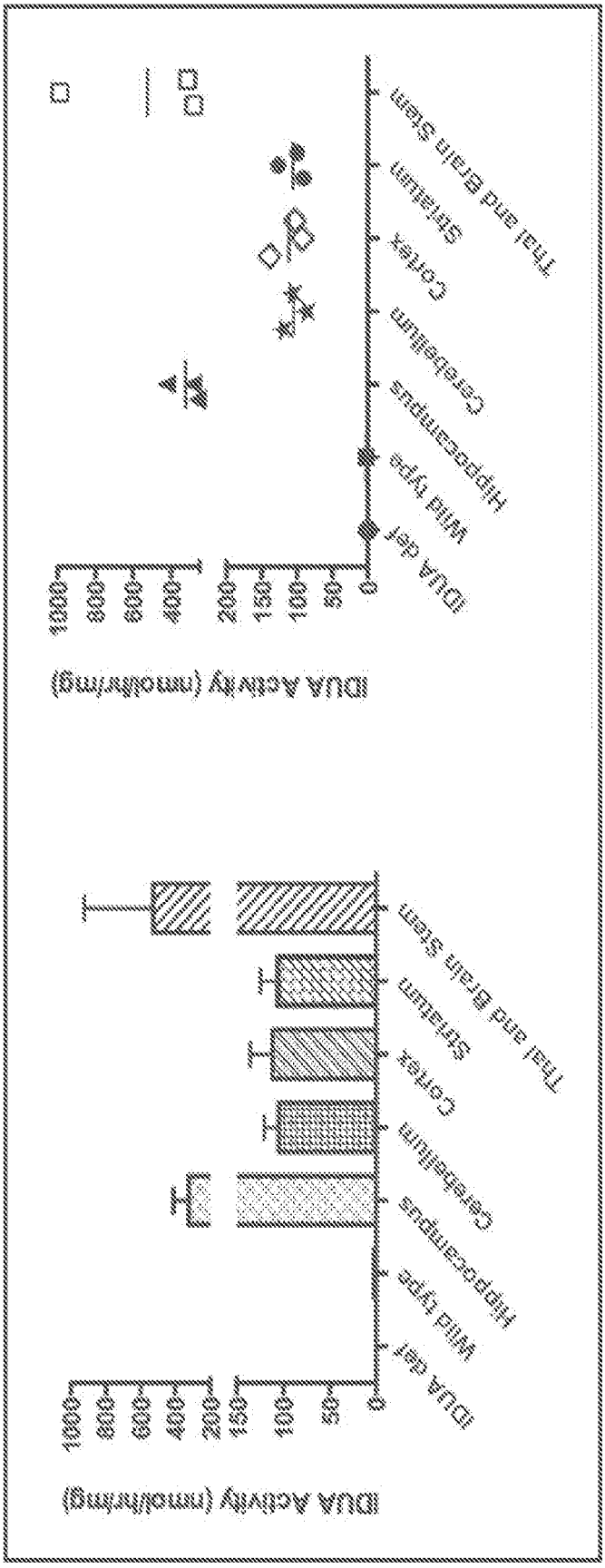


Figure 11. Intracranial Delivery into Immunocompetent mice + Immunosuppression with Cyclophosphamide

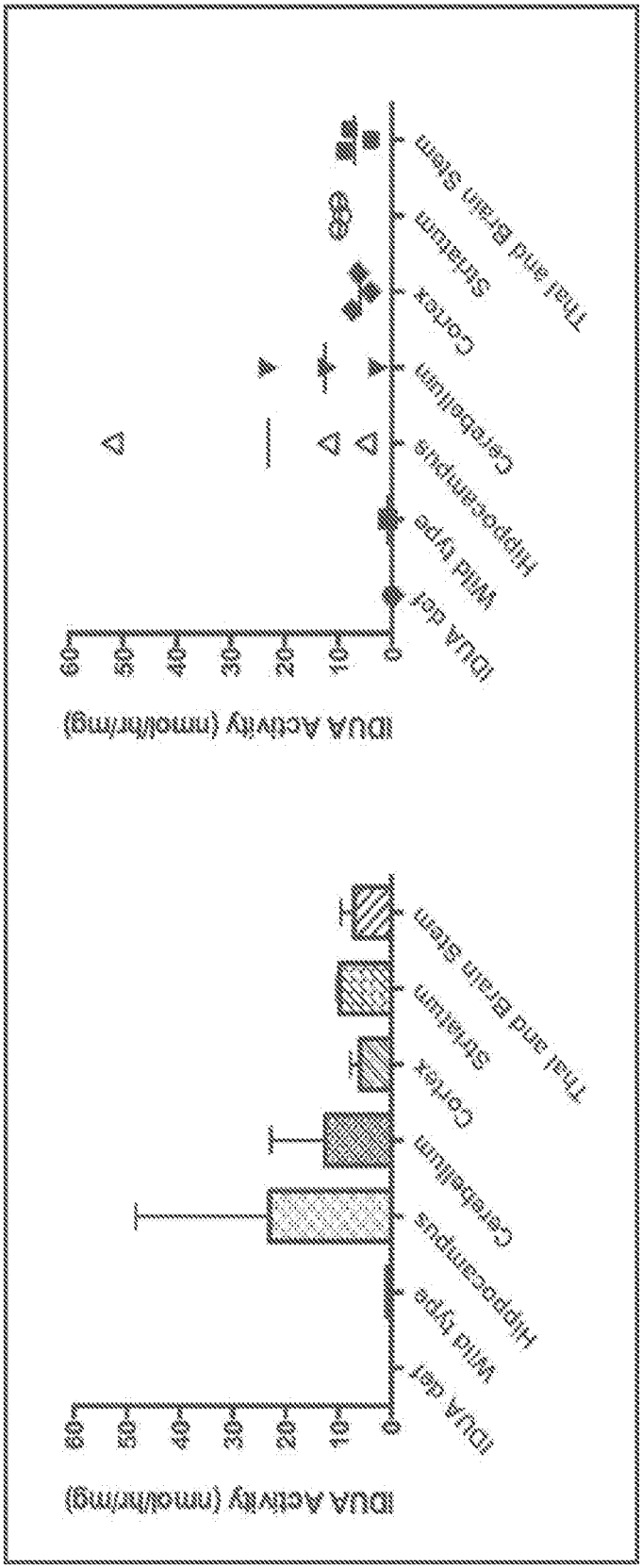


Figure 12. Intracranial Delivery to Immunocompetent mice + Immunotolerization with Aldurazyme

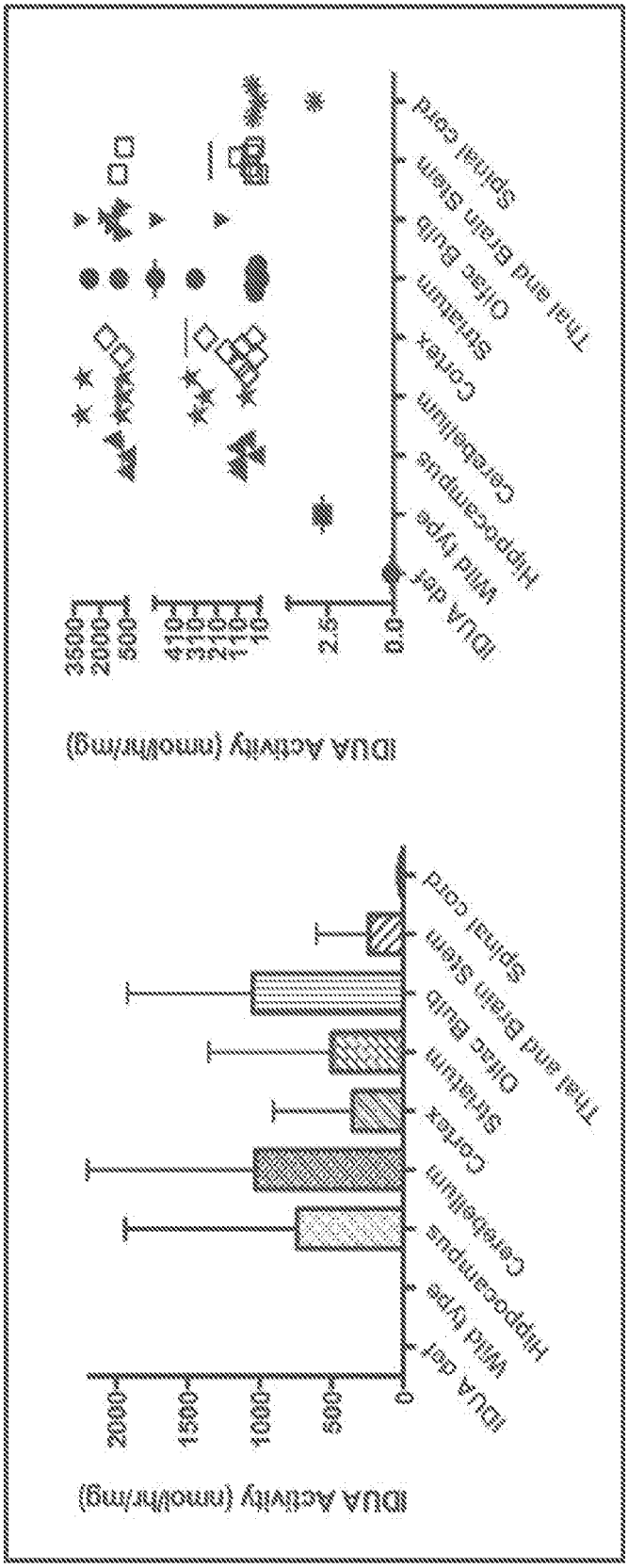


Figure 13. Intrathecal Delivery to Immunocompetent Mice + Immunosuppression with Cyclophosphamide

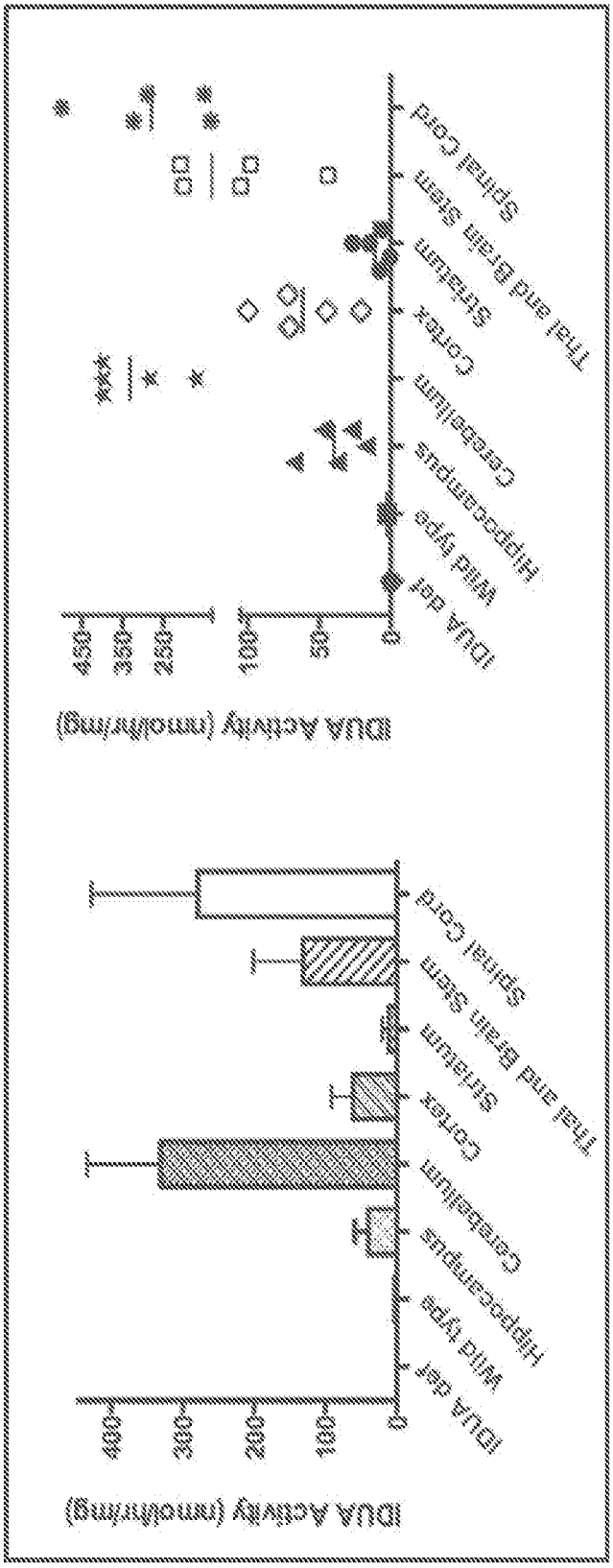


Figure 14. Intrathecal Delivery to Immunocompetent Mice + Immunotolerization with Aldurazyme

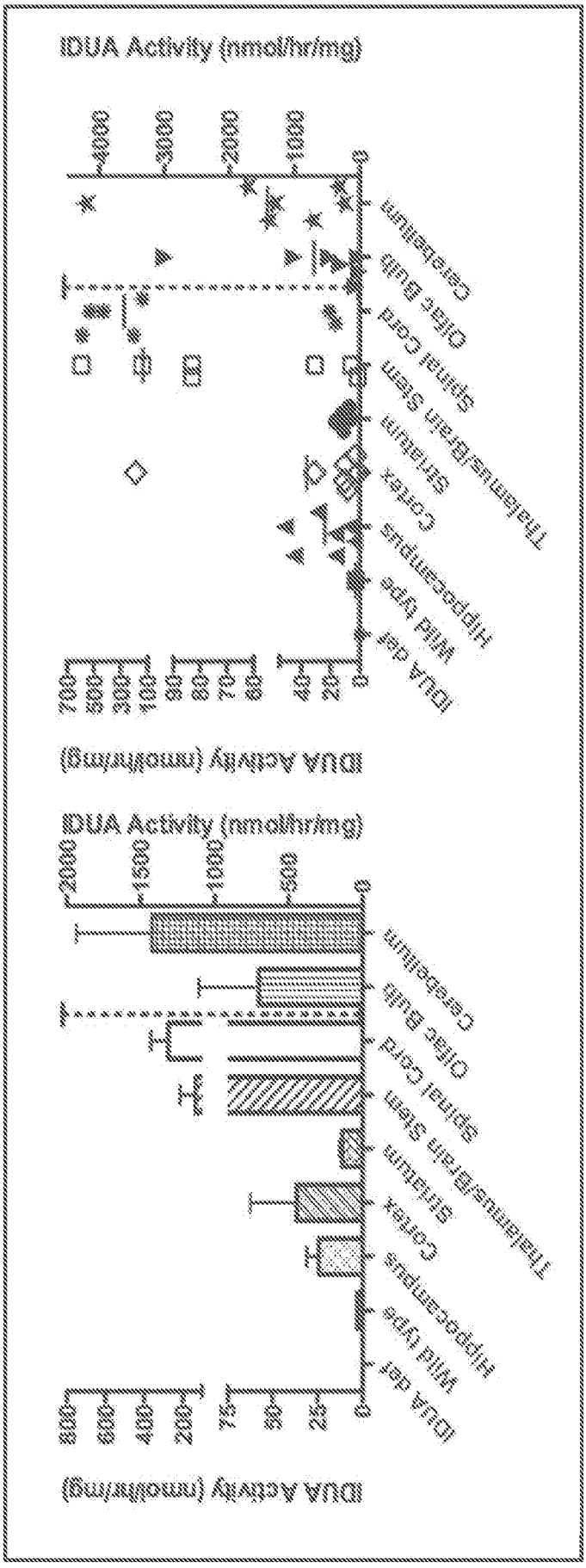


Figure 15. Intrathecal Delivery to Immunocompetent Animals (Controls-No Immunosuppression, No Immunotolerization)

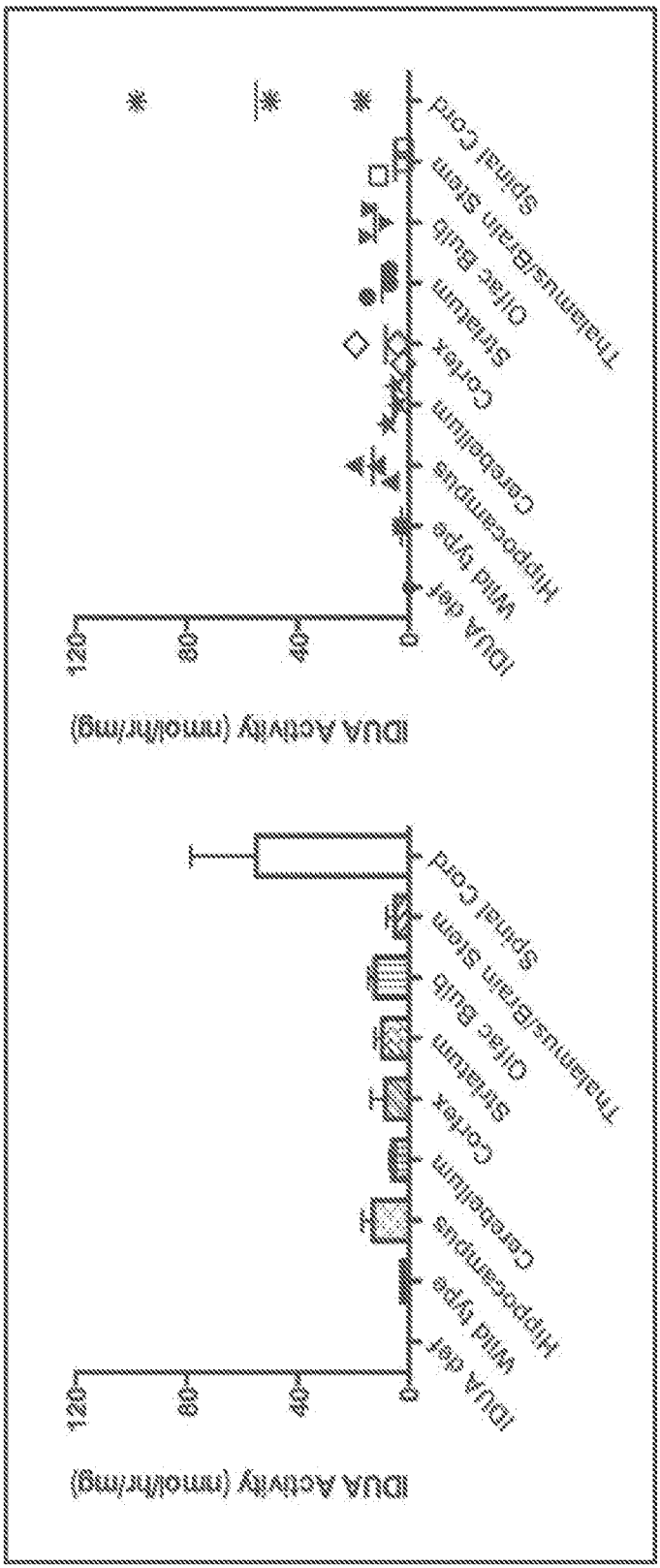


Figure 16. Normalization of Glycosaminoglycans after Intracranial or Intrathecal Administration of AAV9-IDUA

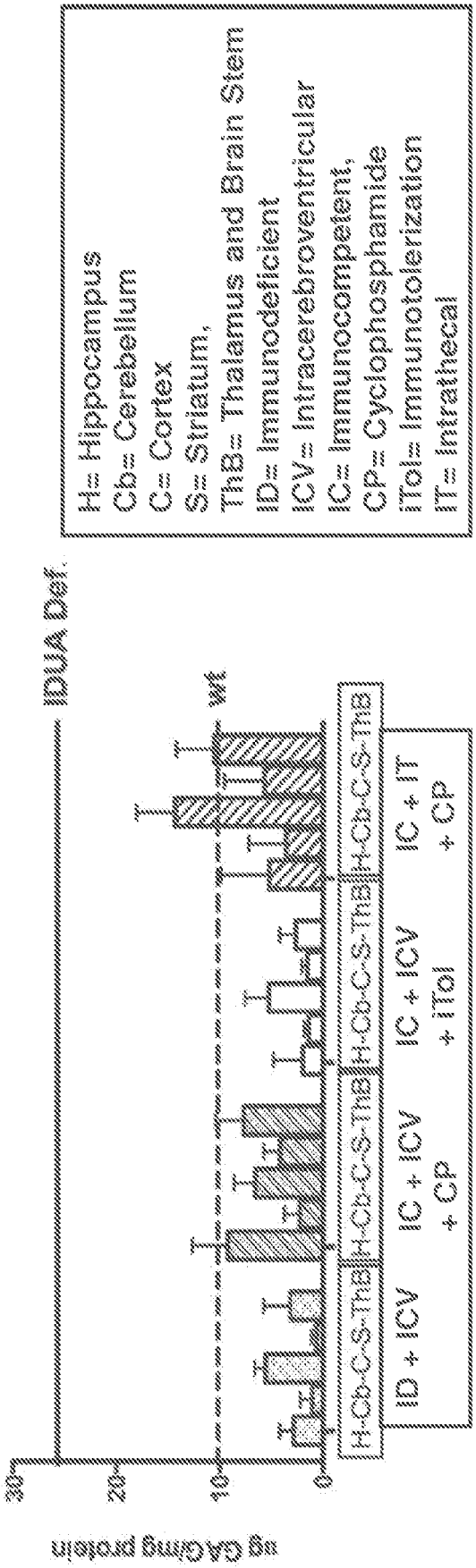
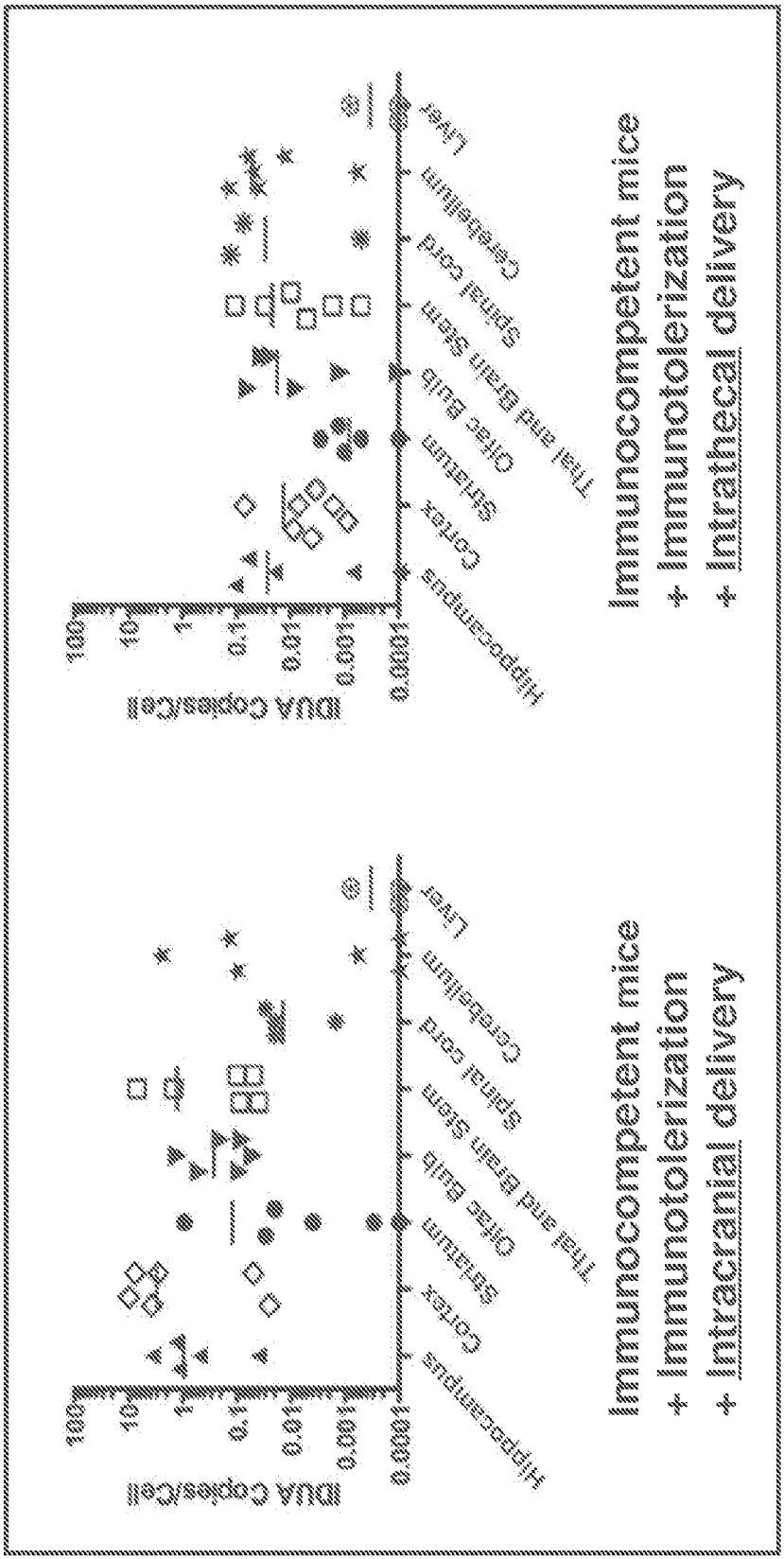


Figure 17. Vector Copy Number in Brain



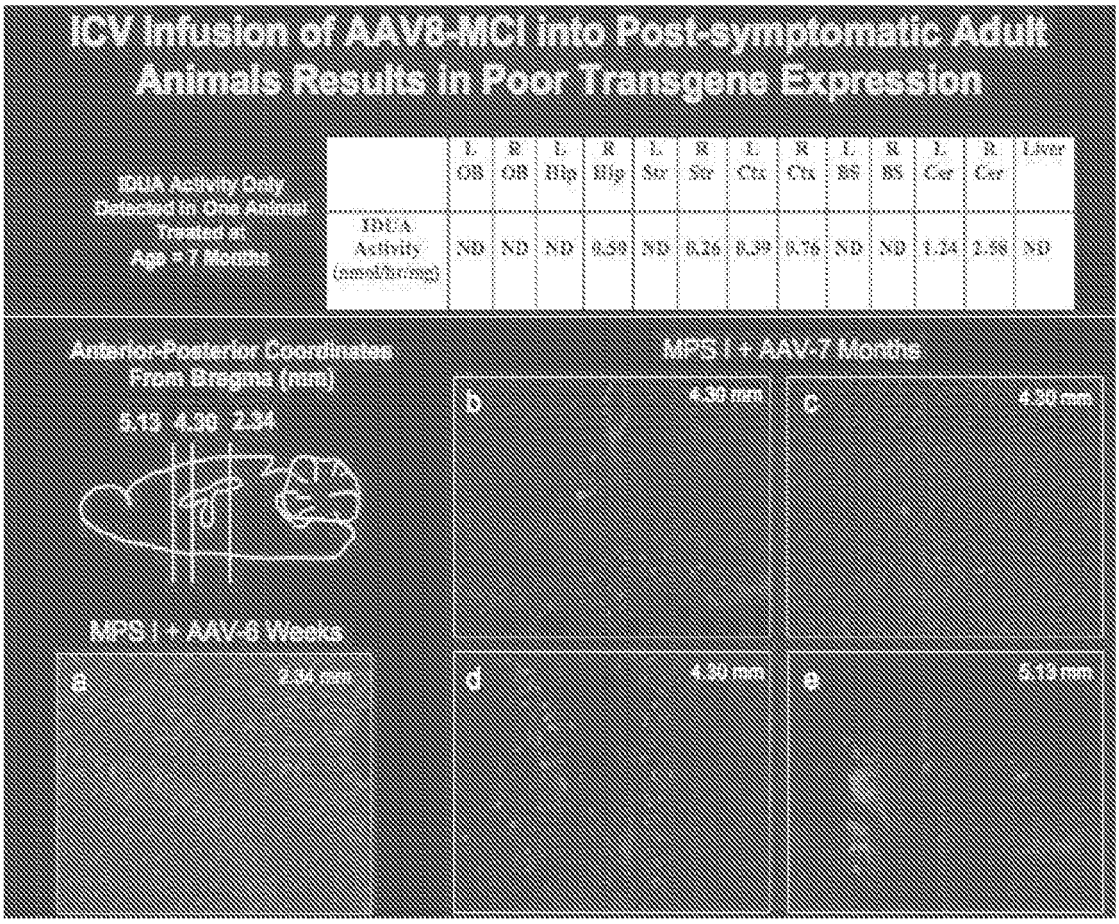

ICV Infusion of AAV8-MCI into Post-symptomatic Adult Animals Results in Poor Transgene Expression
IDUA Activity Only Detected in One Animal Treated at Age = 7 Months
IDUA Activity (nmol/hr/mg)
L GB
R GB
L Hip
R Hip
L Str
R Str
L Ctx
R Ctx
L BS
R BS
L Cer
R Cer
Liver
ND
ND
ND
0.58
ND
0.26
0.39
0.76
ND
ND
1.24
2.66
ND
Anterior-Posterior Coordinates From Bregma (mm)
5.10 4.38 2.34
MPS I + AAV-8 Weeks
a
2.34 mm
MPS I + AAV-7 Months
b
4.38 mm
c
4.38 mm
d
4.38 mm
e
5.10 mm

Figure 18

Figure 19. Intranasal Delivery to Immunocompetent Animals + Immunosuppression with Cyclophosphamide

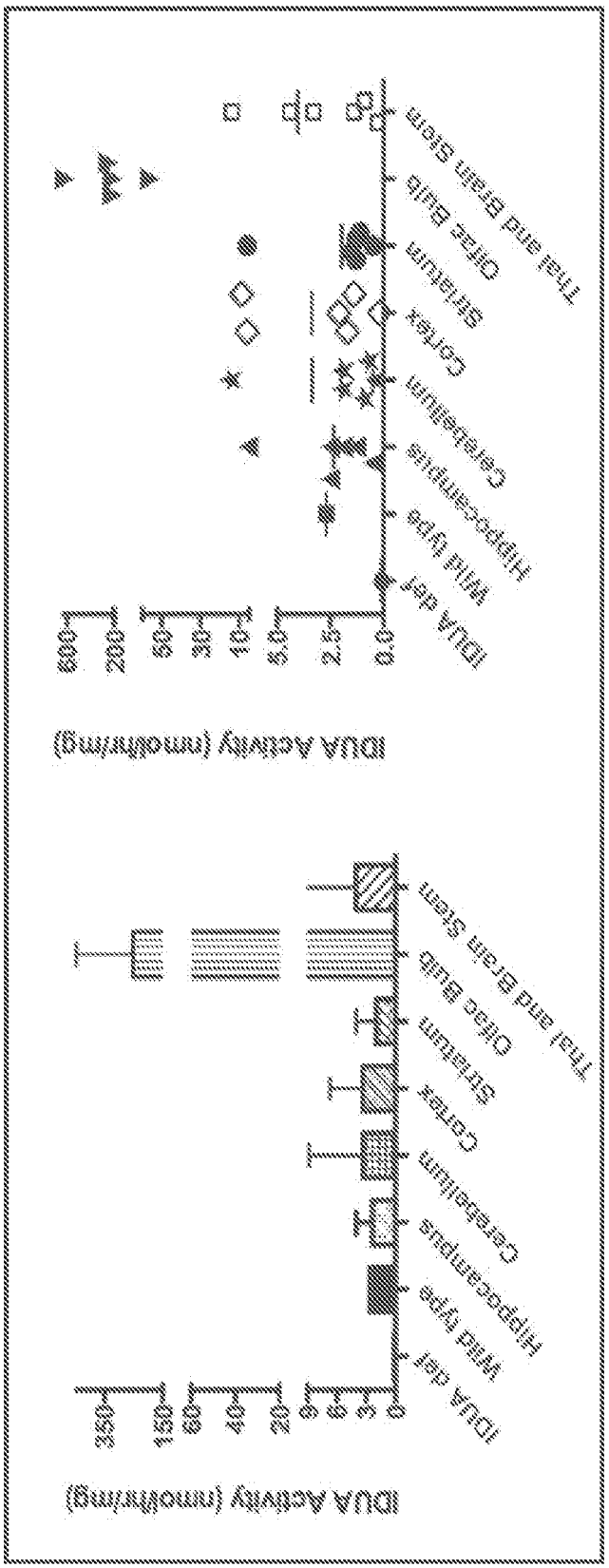


Figure 20. Vector Copy Number in Brain

