Comparison AAV-TT to AAV-2

```
AAVTT   1    MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD   60
             MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD
AAV-2   1    MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD   60

AAVTT   61   KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ   120
             KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AAV-2   61   KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ   120

AAVTT   121  AKKRILEPLGLVEEPVKTAPGKKRPVEHSPAEPDSSSGTGKSGQQPARKRLNFGQTGDAD   180
             AKKR+LEPLGLVEEPVKTAPGKKRPVEHSP EPDSSSGTGKSGQQPARKRLNFGQTGDAD
AAV-2   121  AKKRVLEPLGLVEEPVKTAPGKKRPVEHSPVEPDSSSGTGKAGQQPARKRLNFGQTGDAD   180

AAVTT   181  SVPDPQPLGQPPAAPSGLGTNTMASGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI   240
             SVPDPQPLGQPPAAPSGLGTNTMA GSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI
AAV-2   181  SVPDPQPLGQPPAAPSGLGTNTMATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI   240

AAVTT   241  TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI   300
             TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI
AAV-2   241  TTSTRTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQRLI   300

AAVTT   301  NNNWGFRPKRLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG   360
             NNNWGFRPKRL FKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG
AAV-2   301  NNNWGFRPKRLNFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAHQG   360

AAVTT   361  CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF   420
             CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF
AAV-2   361  CLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVPF   420

AAVTT   421  HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTMSRLQFSQAGASDIRDQSRNWLPG   480
             HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTT SRLQFSQAGASDIRDQSRNWLPG
AAV-2   421  HSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQAGASDIRDQSRNWLPG   480

AAVTT   481  PCYRQQRVSKTAADNNNSDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKYFPQSGVL   540
             PCYRQQRVSKT ADNNNS YSWTGATKYHLNGRDSLVNPGPAMASHKDDEEK FPQSGVL
AAV-2   481  PCYRQQRVSKTSADNNNSEYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVL   540

AAVTT   541  IFGKQDSGKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQSGNTQAATSDVNTQGV   600
             IFGKQ S KTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQ GN QAAT DVNTQGV
AAV-2   541  IFGKQGSEKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQRGNRQAATADVNTQGV   600
```

FIG. 1

Comparison AAV-TT to AAV-9

```
AAVTT   1    MAADGYLPDWLEDTLSEGIRQWWKLKPGPPPPKPAERHKDDSRGLVLPGYKYLGPFNGLD  60
             MAADGYLPDWLED LSEGIR+WW LKPG P PK  ++H+D++RGLVLPGYKYLGP NGLD
AAV-9   1    MAADGYLPDWLEDNLSEGIREWWALKPGAPQPKANQQHQDNARGLVLPGYKYLGPGNGLD  60

AAVTT   61   KGEPVNEADAAALEHDKAYDRQLDSGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ  120
             KGEPVN ADAAALEHDKAYD+QL +GDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ
AAV-9   61   KGEPVNAADAAALEHDKAYDQQLKAGDNPYLKYNHADAEFQERLKEDTSFGGNLGRAVFQ  120

AAVTT   121  AKKRILEPLGLVEEPVKTAPGKKRPVEHSPAEPDSSSGTGKSGQQPARKRLNFGQTGDAD  180
             AKKR+LEPLGLVEE  KTAPGKKRPVE SP EPDSS+G GKSG QPA+KRLNFGQTGD +
AAV-9   121  AKKRLLEPLGLVEEAAKTAPGKKRPVEQSPQEPDSSAGIGKSGAQPAKKRLNFGQTGDTE  180

AAVTT   181  SVPDPQPLGQPPAAPSGLGTNTMASGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVI  240
             SVPDPQP+G+PPAAPSG+G+ TMASG GAP+ADNNEGADGVG+SSGNWHCDS W+GDRVI
AAV-9   181  SVPDPQPIGEPPAAPSGVGSLTMASGGGAPVADNNEGADGVGSSSGNWHCDSQWLGDRVI  240

AAVTT   241  TTSTRTWALPTYNNHLYKQI--SSQSGASNDNHYFGYSTPWGYFDFNRFHCHFSPRDWQR  298
             TTSTRTWALPTYNNHLYKQI  S+  G+SNDN YFGYSTPWGYFDFNRFHCHFSPRDWQR
AAV-9   241  TTSTRTWALPTYNNHLYKQISNSTSGGSSNDNAYFGYSTPWGYFDFNRFHCHFSPRDWQR  300

AAVTT   299  LINNNWGFRPKRLSFKLFNIQVKEVTQNDGTTTIANNLTSTVQVFTDSEYQLPYVLGSAH  358
             LINNNWGFRPKRL+FKLFNIQVKEVT N+G  TIANNLTSTVQVFTDS+YQLPYVLGSAH
AAV-9   301  LINNNWGFRPKRLNFKLFNIQVKEVTDNNGVKTIANNLTSTVQVFTDSDYQLPYVLGSAH  360

AAVTT   359  QGCLPPFPADVFMVPQYGYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDV  418
             +GCLPPFPADVFM+PQYGYLTLN+GSQAVGRSSFYCLEYFPSQMLRTGNNF FSY FE+V
AAV-9   361  EGCLPPFPADVFMIPQYGYLTLNDGSQAVGRSSFYCLEYFPSQMLRTGNNFQFSYEFENV  420

AAVTT   419  PFHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTMSRLQFSQAGASDIRDQSRNWL  478
             PFHSSYAHSQSLDRLMNPLIDQYLYYLS+T   SG    + L+FS AG S++  Q RN++
AAV-9   421  PFHSSYAHSQSLDRLMNPLIDQYLYYLSKTINGSGQNQQT-LKFSVAGPSNMAVQGRNYI  479

AAVTT   479  PGPCYRQQRVSKTAADNNNSDYSWTGATKYHLNGRDSLVNPGPAMASHKDDEEKYFPQSG  538
             PGP YRQQRVS T   NNNS+++W GA+ + LNGR+SL+NPGPAMASHK+ E+++FP SG
AAV-9   480  PGPSYRQQRVSTTVTQNNNSEFAWPGASSWALNGRNSLMNPGPAMASHKEGEDRFFPLSG  539

AAVTT   539  VLIFGKQDSGKTNVDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQSGNTQAATSDVNTQ  598
             +LIFGKQ +G+ NVD +KVMIT+EEEI+TTNPVATE YG V+TN QS   QA T  V  Q
AAV-9   540  SLIFGKQGTGRDNVDADKVMITNEEEIKTTNPVATESYGQVATNHQSAQAQAQTGWVQNQ  599

AAVTT   599  GVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFGLKHPPPQILIKNTPVPANPS  658
```

FIG. 2

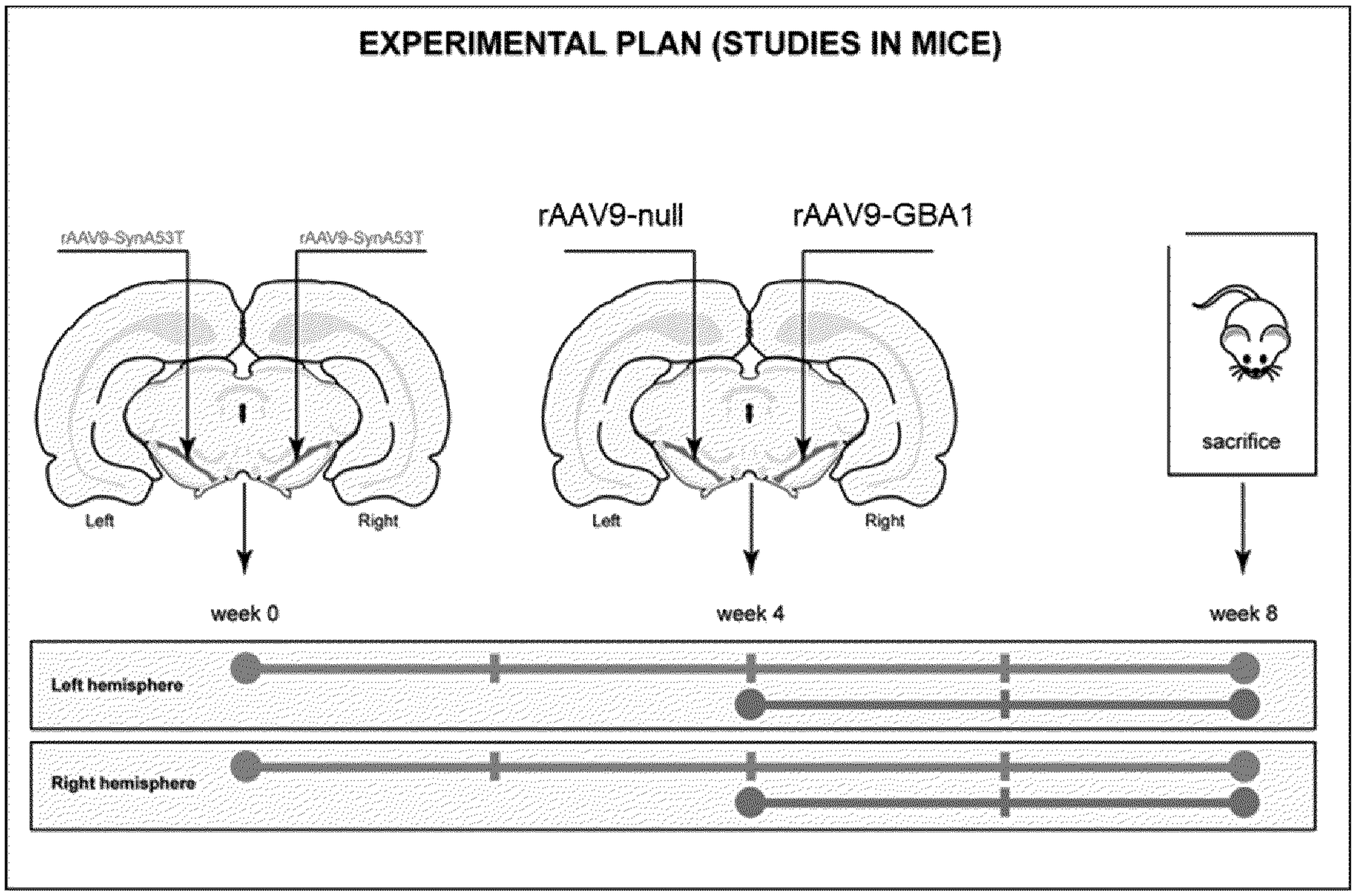

EXPERIMENTAL PLAN (STUDIES IN MICE)
rAAV9-SynA53T
rAAV9-SynA53T
rAAV9-null
rAAV9-GBA1
Left
Right
Left
Right
sacrifice
week 0
week 4
week 8
Left hemisphere
Right hemisphere

FIG. 3

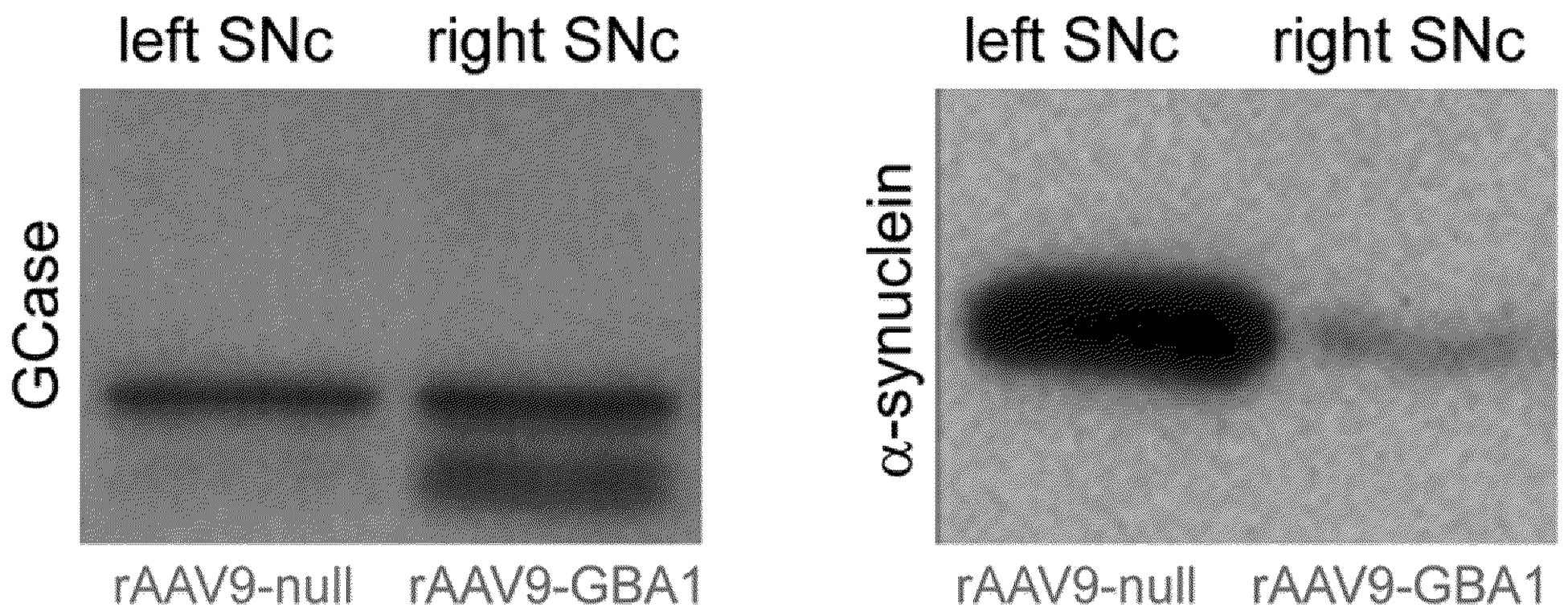

left SNc
right SNc
GCase
left SNc
right SNc
α-synuclein
rAAV9-null
rAAV9-GBA1
rAAV9-null
rAAV9-GBA1

FIG. 4

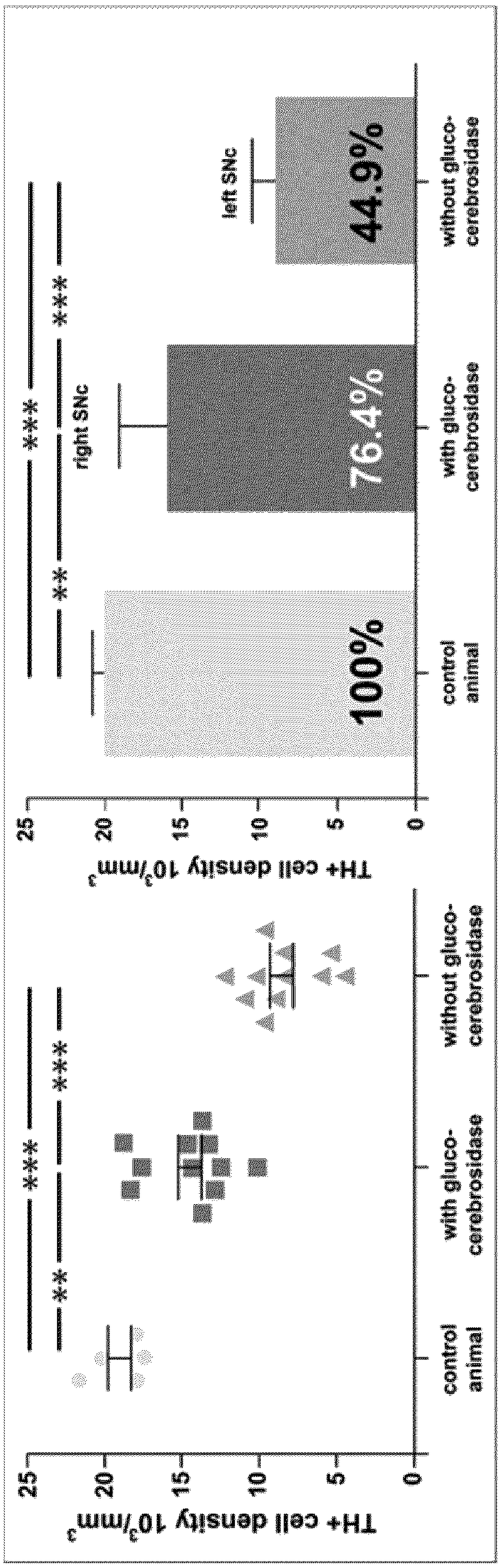

TH+ cell density 10³/mm³
25
20
15
10
5
0
right SNc
left SNc
100%
76.4%
44.9%
***
**
***
***
control animal
with gluco-cerebrosidase
without gluco-cerebrosidase

FIG. 5

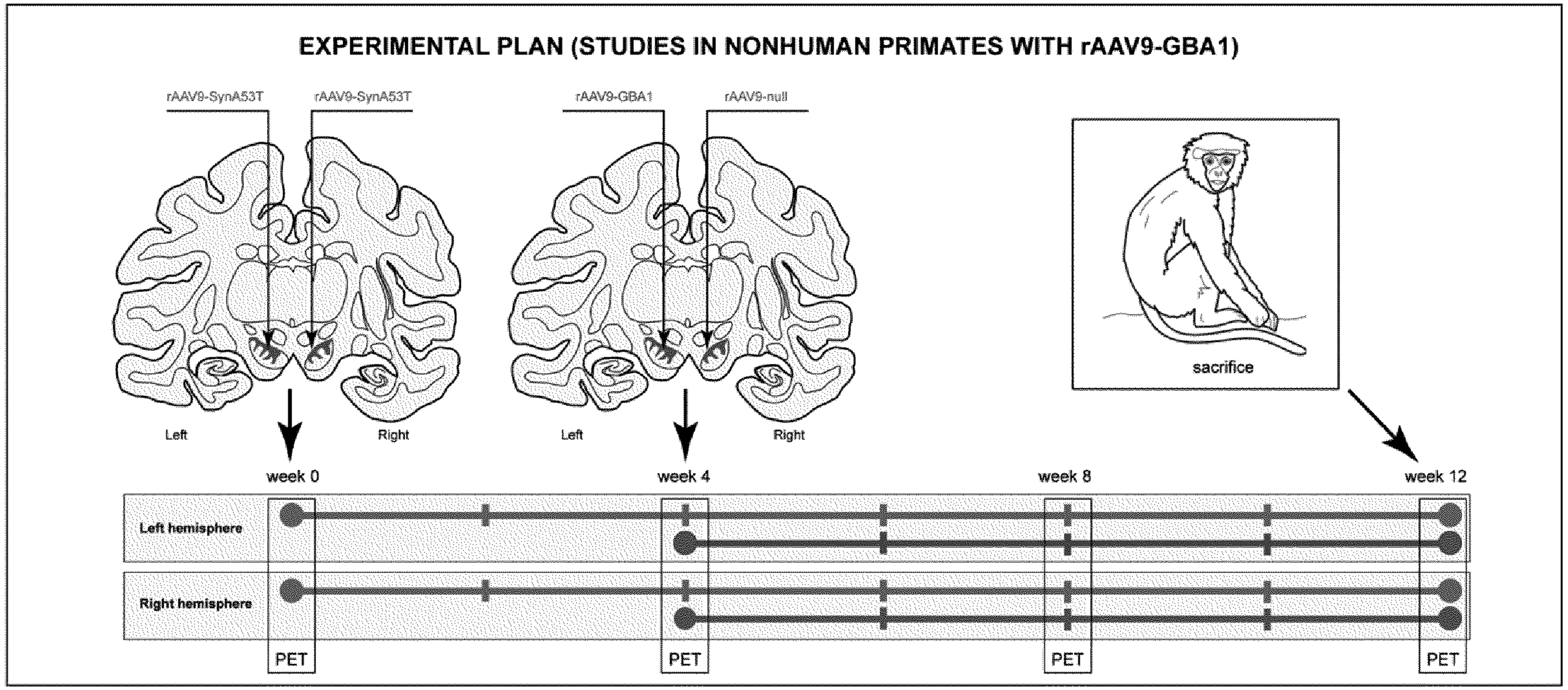

EXPERIMENTAL PLAN (STUDIES IN NONHUMAN PRIMATES WITH rAAV9-GBA1)
rAAV9-SynA53T
rAAV9-SynA53T
rAAV9-GBA1
rAAV9-null
Left
Right
Left
Right
sacrifice
week 0
week 4
week 8
week 12
Left hemisphere
Right hemisphere
PET
PET
PET
PET

FIG. 6

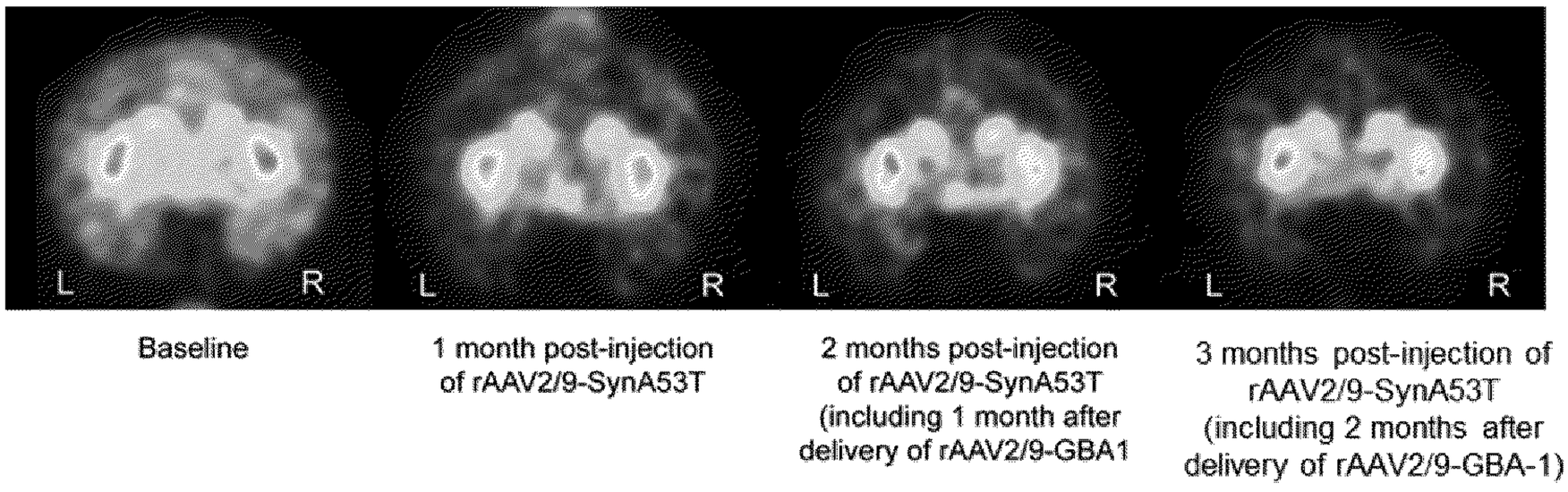

L
R
L
R
L
R
L
R
Baseline
1 month post-injection of rAAV2/9-SynA53T
2 months post-injection of rAAV2/9-SynA53T (including 1 month after delivery of rAAV2/9-GBA1
3 months post-injection of rAAV2/9-SynA53T (including 2 months after delivery of rAAV2/9-GBA-1)

FIG. 7

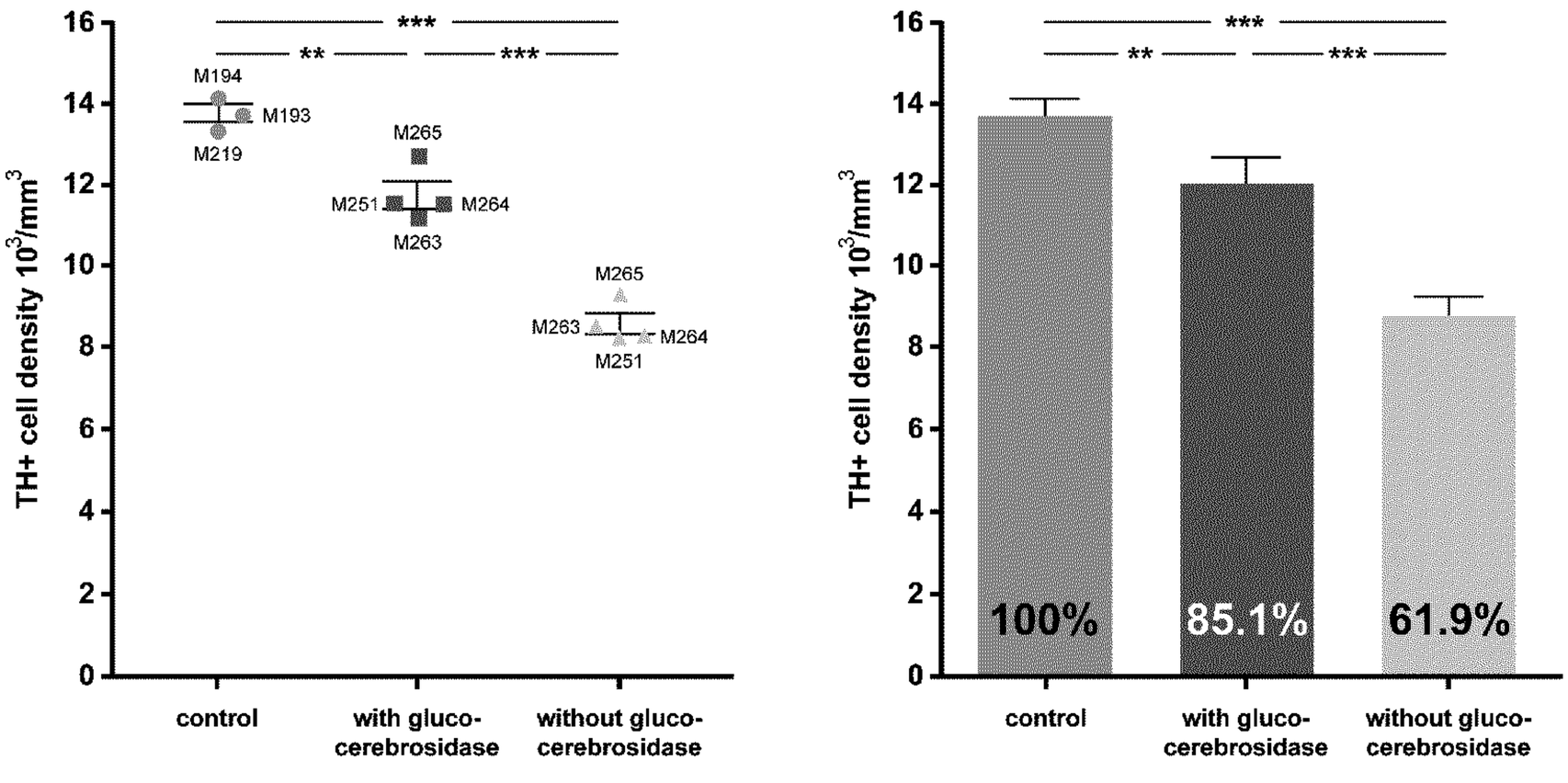

TH+ cell density 10^3/mm^3
16
14
12
10
8
6
4
2
0
***
**
***
M194
M193
M219
M265
M251
M264
M263
M265
M263
M264
M251
control
with gluco-cerebrosidase
without gluco-cerebrosidase
TH+ cell density 10^3/mm^3
***
**
***
100%
85.1%
61.9%
control
with gluco-cerebrosidase
without gluco-cerebrosidase

FIG. 8

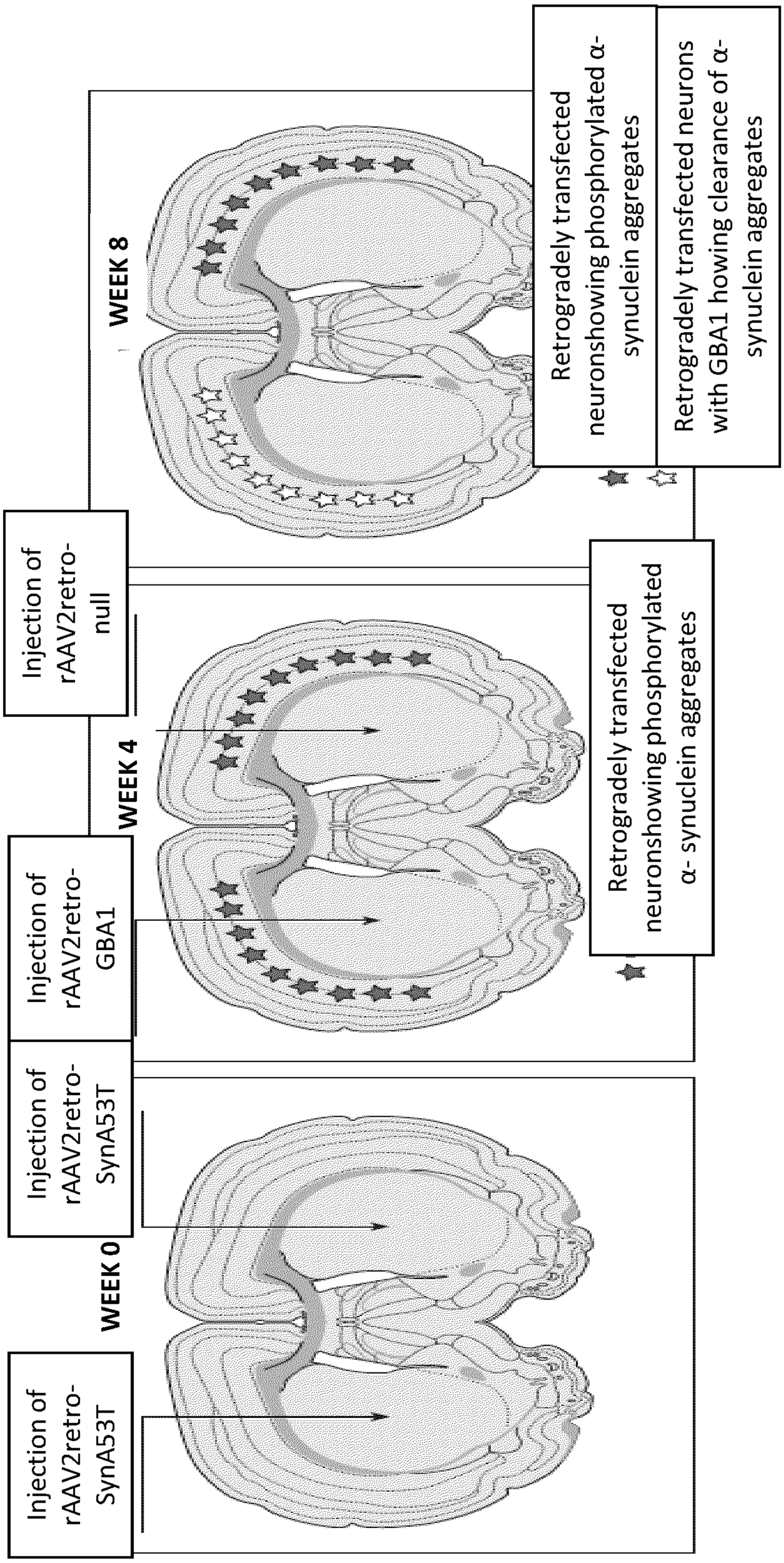

Injection of rAAV2retro-SynA53T
WEEK 0
Injection of rAAV2retro-GBA1
WEEK 4
Injection of rAAV2retro-null
WEEK 8
Retrogradely transfected neuronshowing phosphorylated α- synuclein aggregates
Retrogradely transfected neuronshowing phosphorylated synuclein aggregates
Retrogradely transfected neurons with GBA1 howing clearance of α-synuclein aggregates

FIG. 9

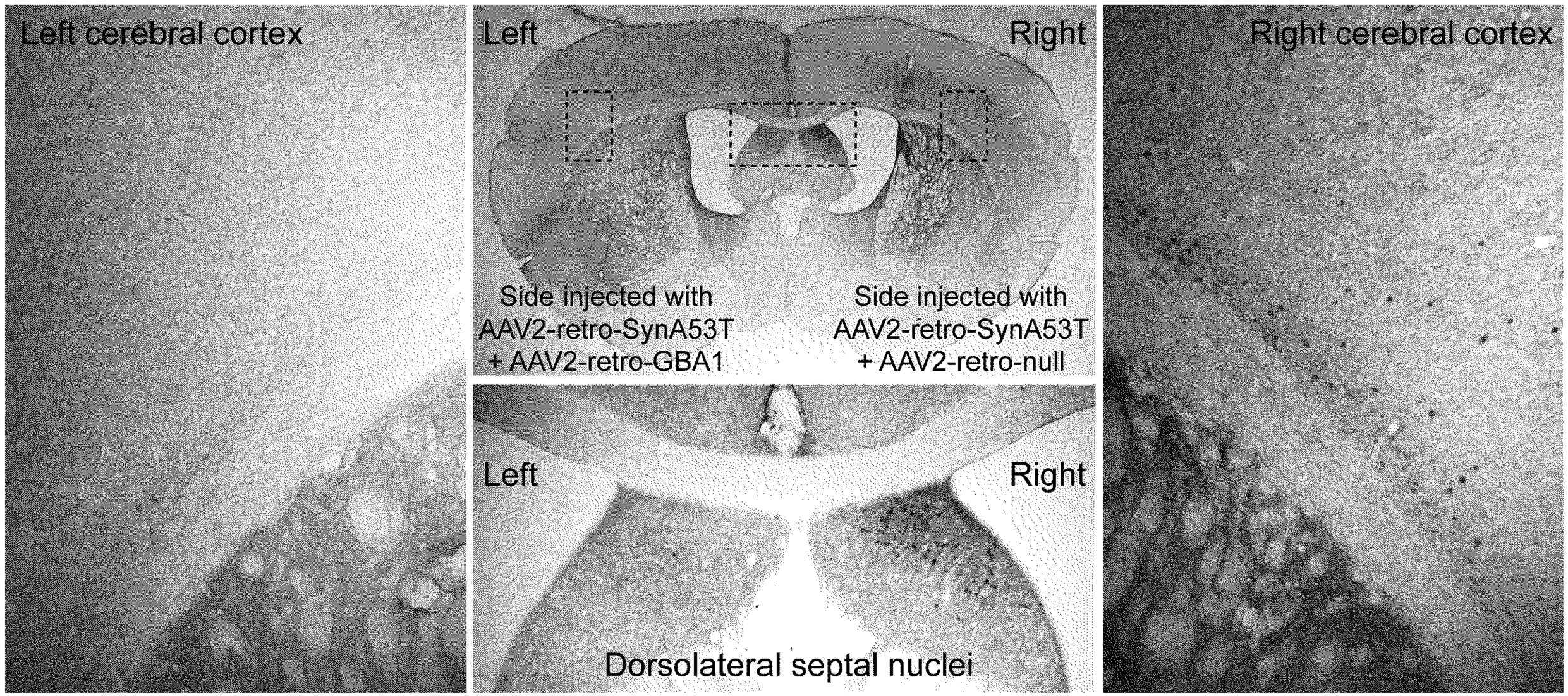

Left cerebral cortex
Left
Right
Side injected with
AAV2-retro-SynA53T
+ AAV2-retro-GBA1
Side injected with
AAV2-retro-SynA53T
+ AAV2-retro-null
Right cerebral cortex
Left
Right
Dorsolateral septal nuclei

FIG. 10

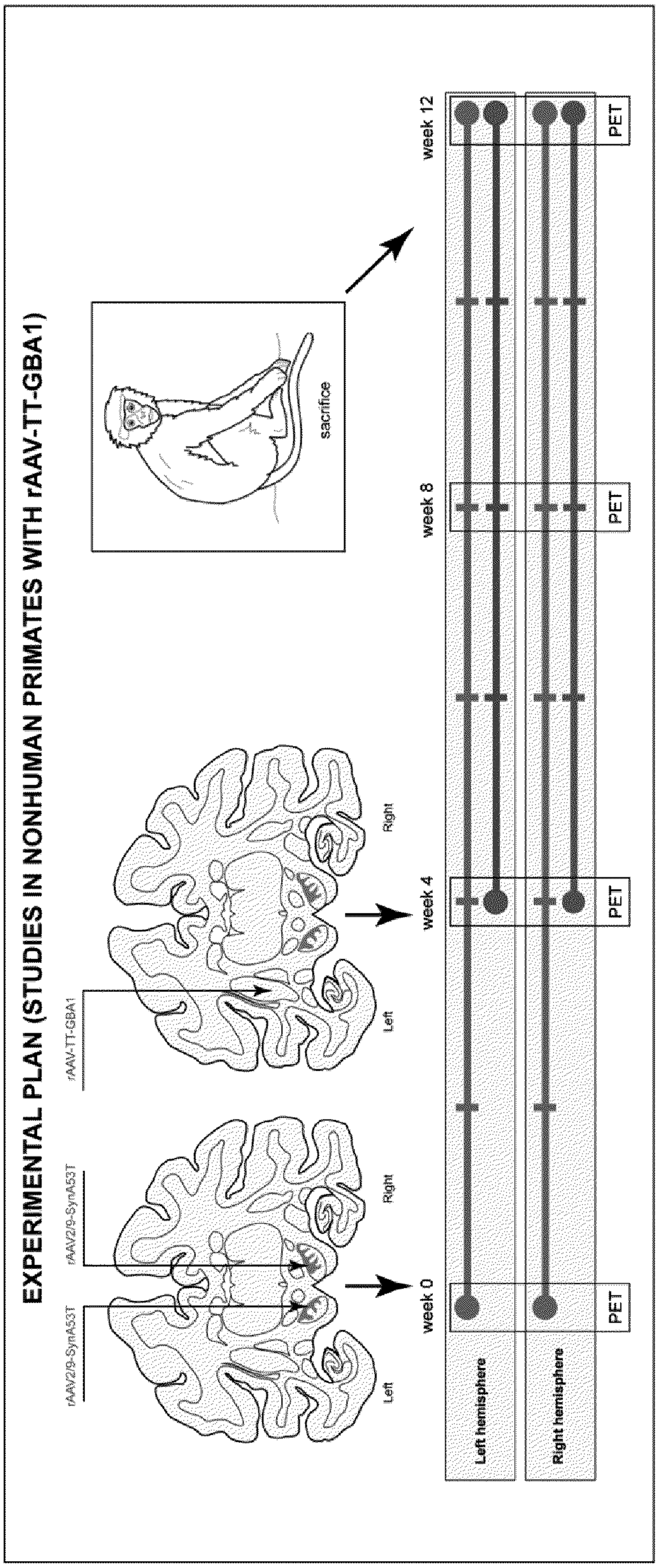
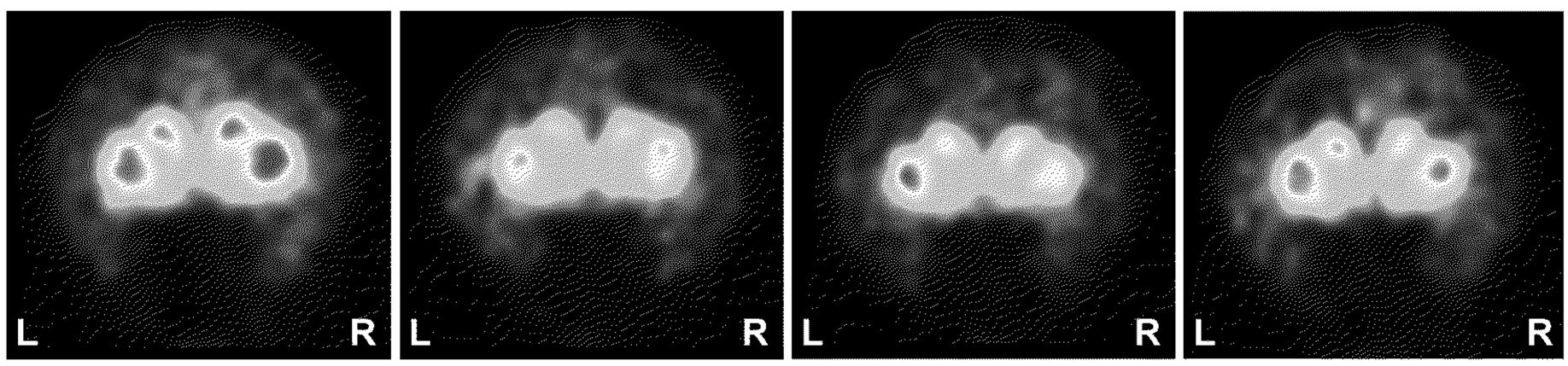

EXPERIMENTAL PLAN (STUDIES IN NONHUMAN PRIMATES WITH rAAV-TT-GBA1)
rAAV2/9-SynA53T
rAAV-TT-GBA1
Right
Left
week 0
week 4
week 8
week 12
sacrifice
PET
Left hemisphere
Right hemisphere

FIG. 11

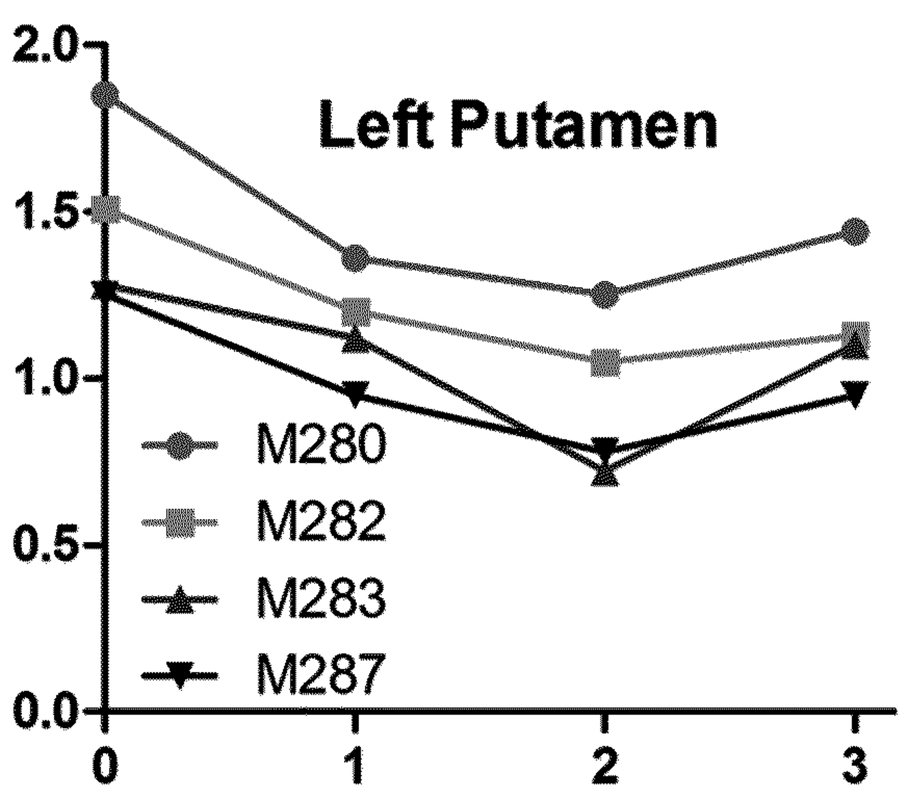


baseline

1 month post-injection of rAAV9-SynA53T

2 months post-injection of rAAV9-SynA53T (including 0.5 months after delivery of rAAV-tt-GBA1)

3 months post-injection of rAAV9-SynA53T (including 1.5 months after delivery of rAAV-tt-GBA1)



Post-commissural putamen
(quatification of binding potential)

FIG. 12

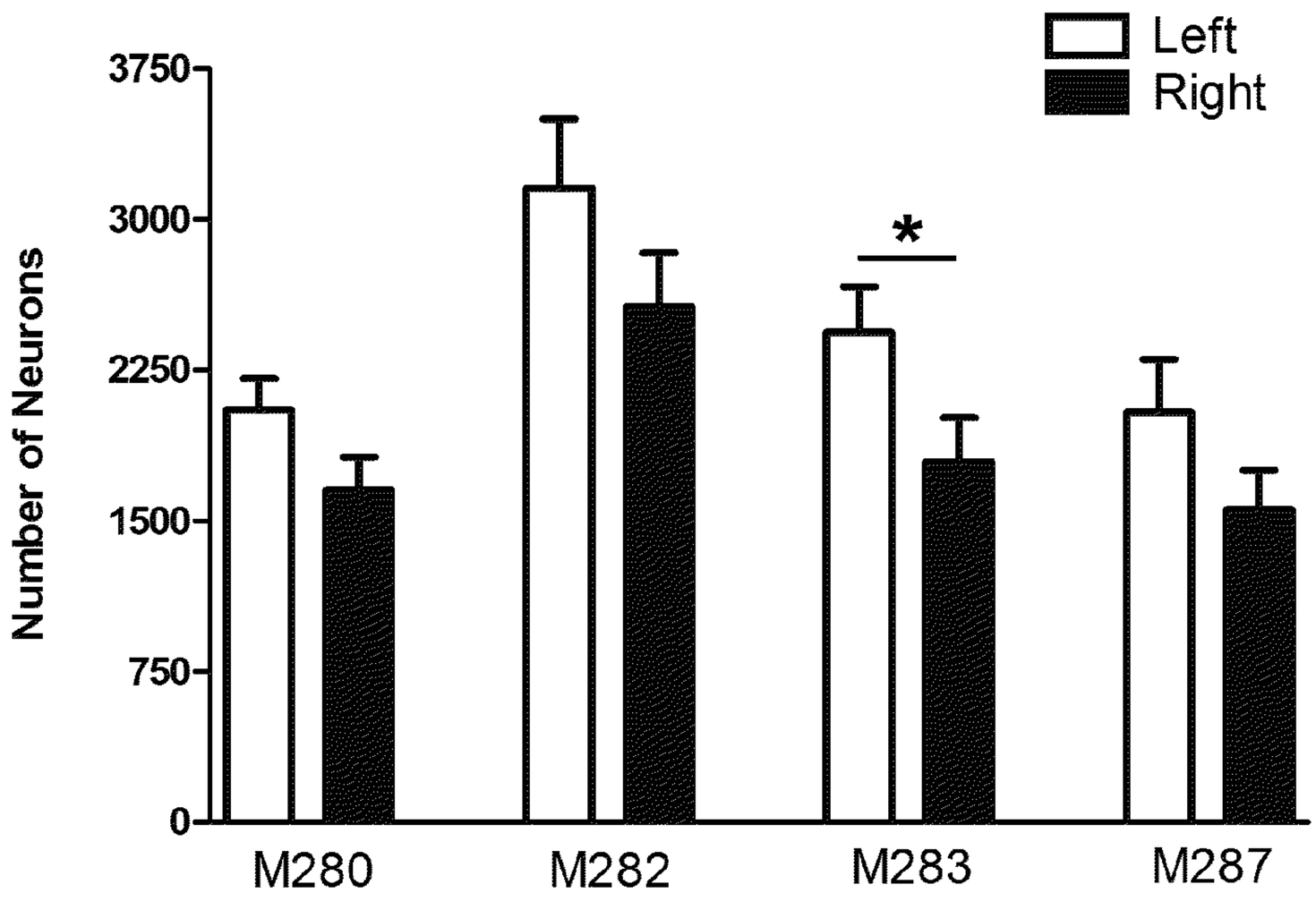

Left
Right
Number of Neurons
3750
3000
2250
1500
750
0
*
M280
M282
M283
M287

FIG. 13

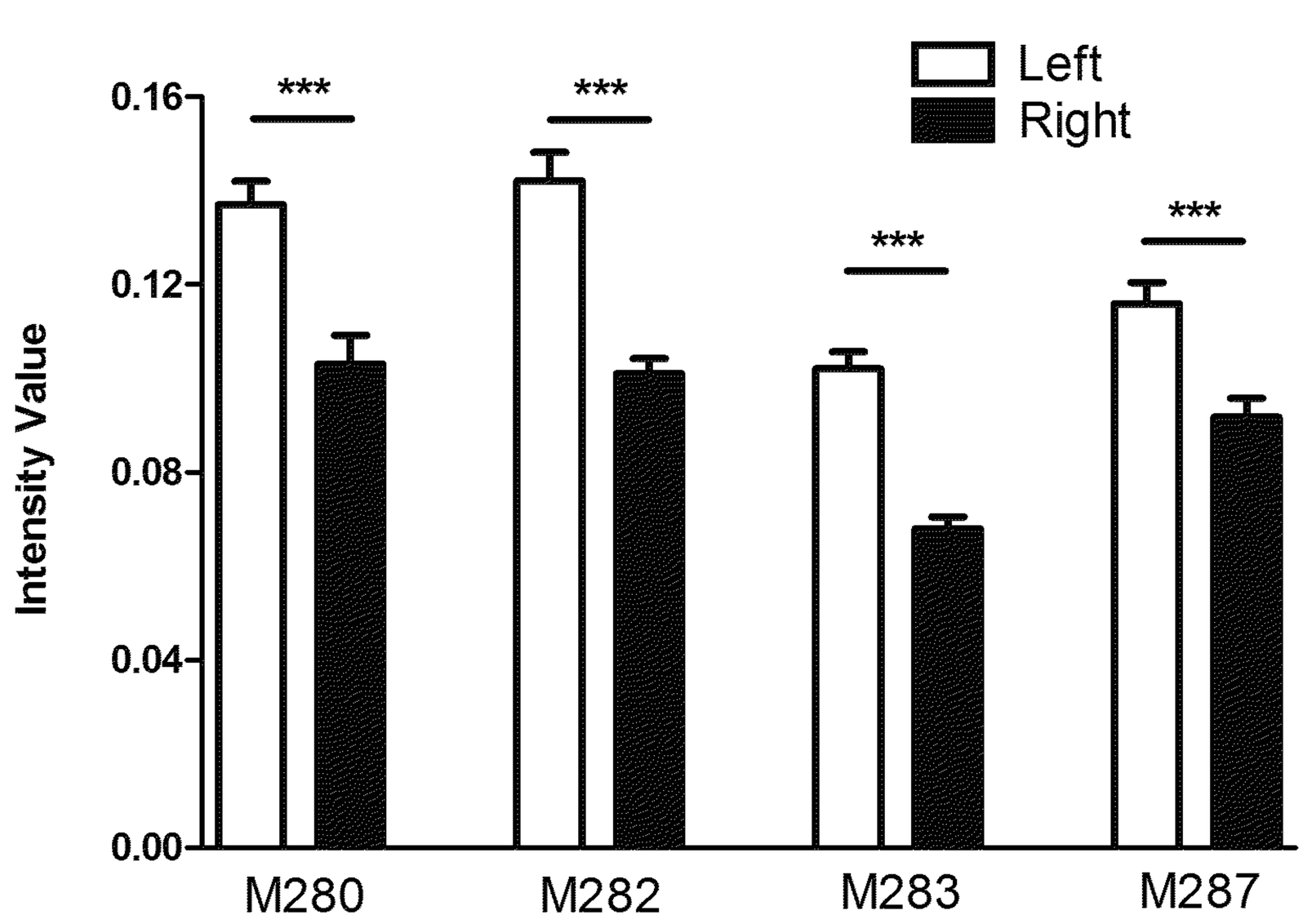

Left
Right
Intensity Value
0.16
0.12
0.08
0.04
0.00
***
***
***
***
M280
M282
M283
M287

FIG. 14

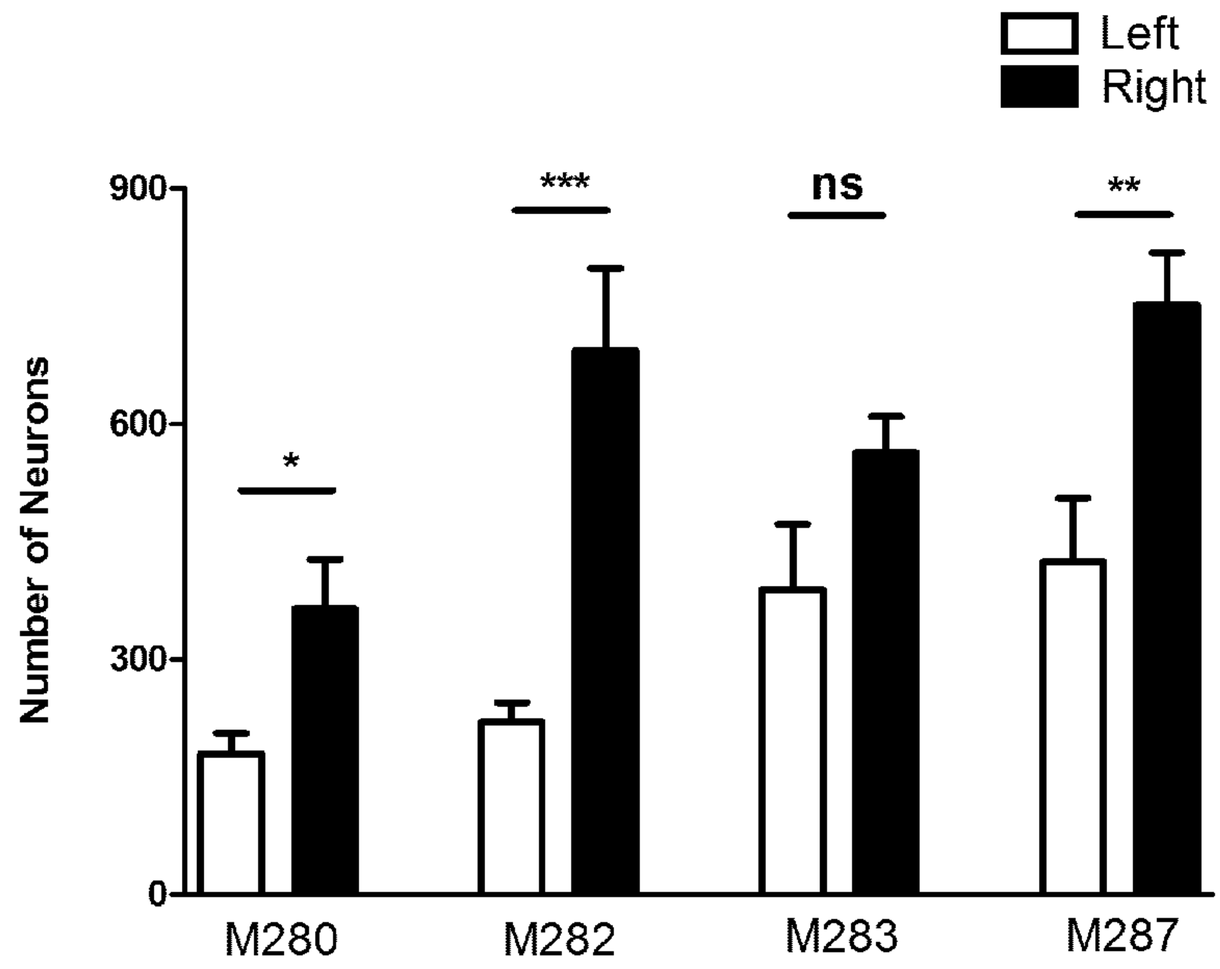

Left
Right
900
600
300
0
Number of Neurons
*
***
ns
**
M280
M282
M283
M287

FIG. 15

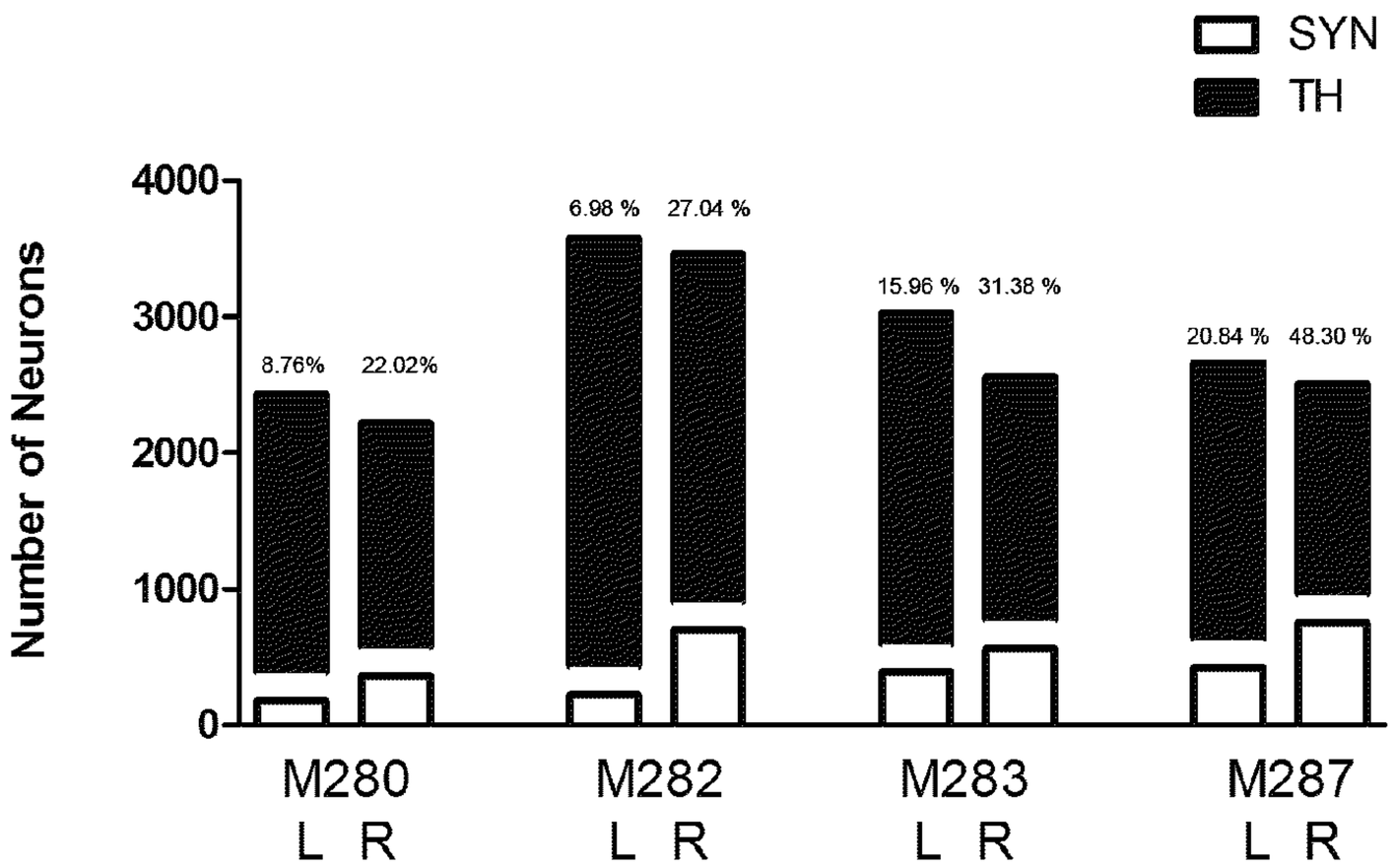

SYN
TH
4000
3000
2000
1000
0
Number of Neurons
8.76%
22.02%
6.98 %
27.04 %
15.96 %
31.38 %
20.84 %
48.30 %
M280
M282
M283
M287
L R
L R
L R
L R

FIG. 16

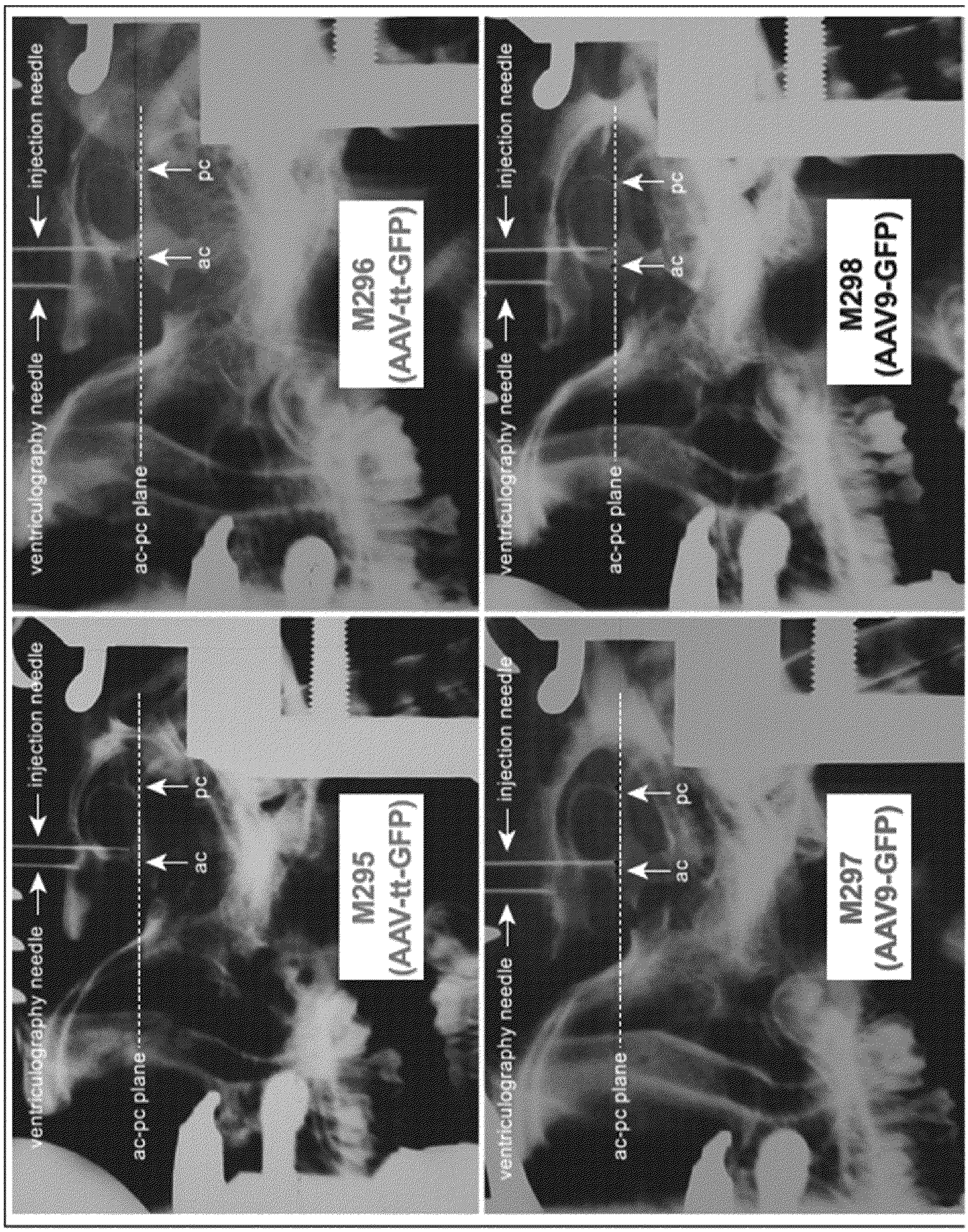

ventriculography needle
injection needle
ac-pc plane
ac
pc
M296
(AAV-tt-GFP)
M298
(AAV9-GFP)
M295
(AAV-tt-GFP)
M297
(AAV9-GFP)

FIG. 17

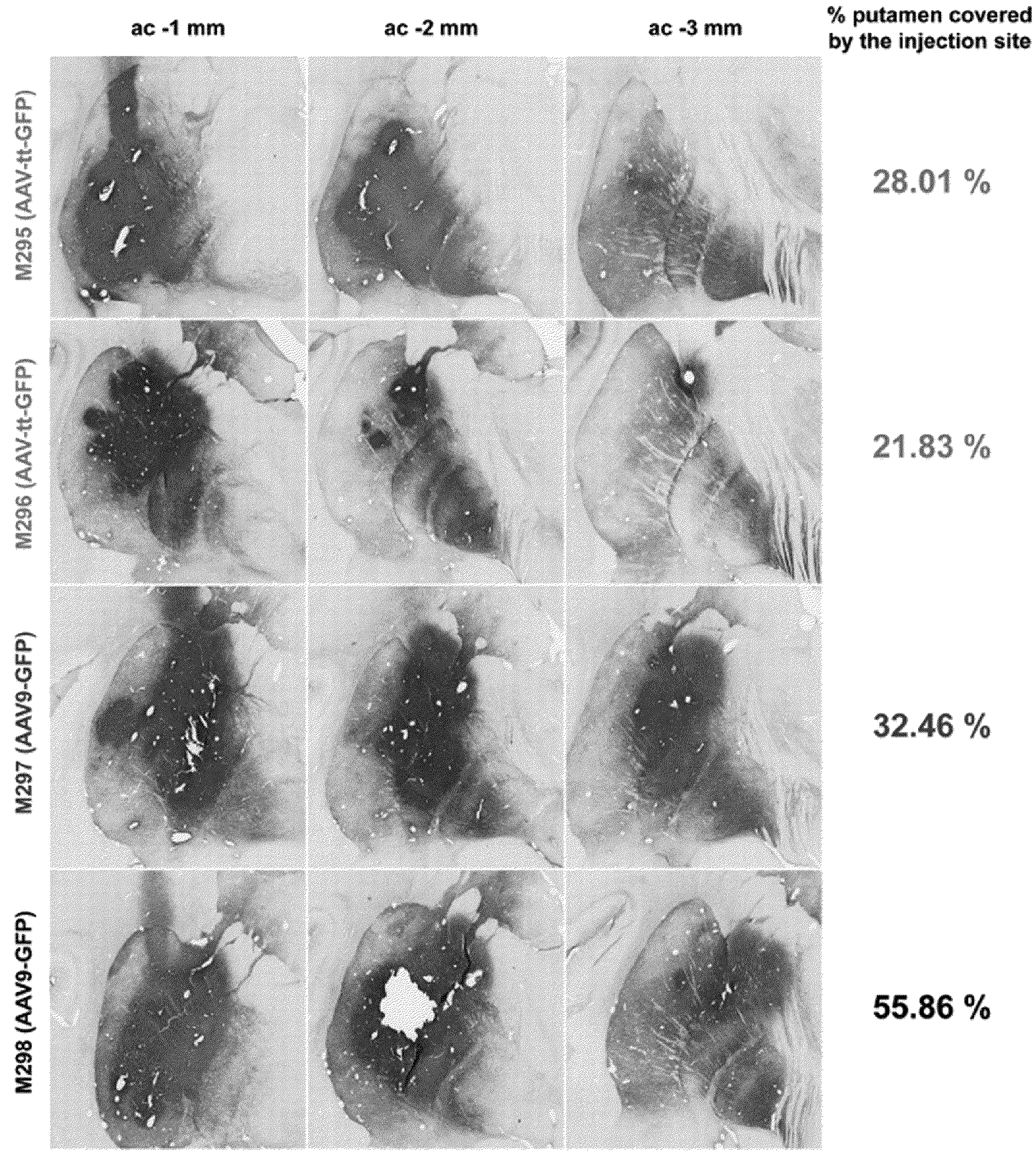

ac -1 mm
ac -2 mm
ac -3 mm
% putamen covered by the injection site
M295 (AAV-tt-GFP)
28.01 %
M296 (AAV-tt-GFP)
21.83 %
M297 (AAV9-GFP)
32.46 %
M298 (AAV9-GFP)
55.86 %

FIG. 18

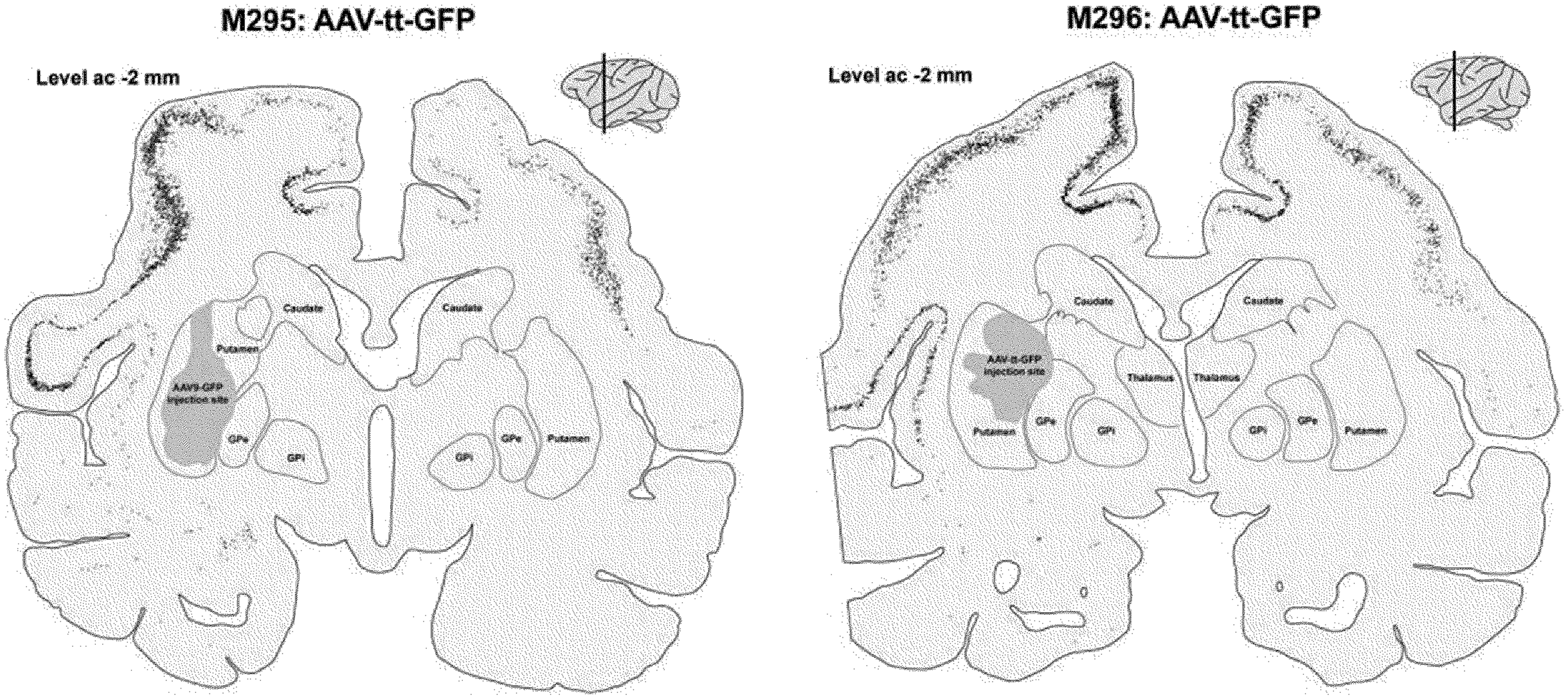

M295: AAV-tt-GFP
Level ac -2 mm
Caudate
Caudate
Putamen
AAV9-GFP injection site
GPe
GPi
GPi
GPe
Putamen
M296: AAV-tt-GFP
Level ac -2 mm
Caudate
Caudate
AAV-tt-GFP injection site
Thalamus
Thalamus
Putamen
GPe
GPi
GPi
GPe
Putamen

FIG. 19A

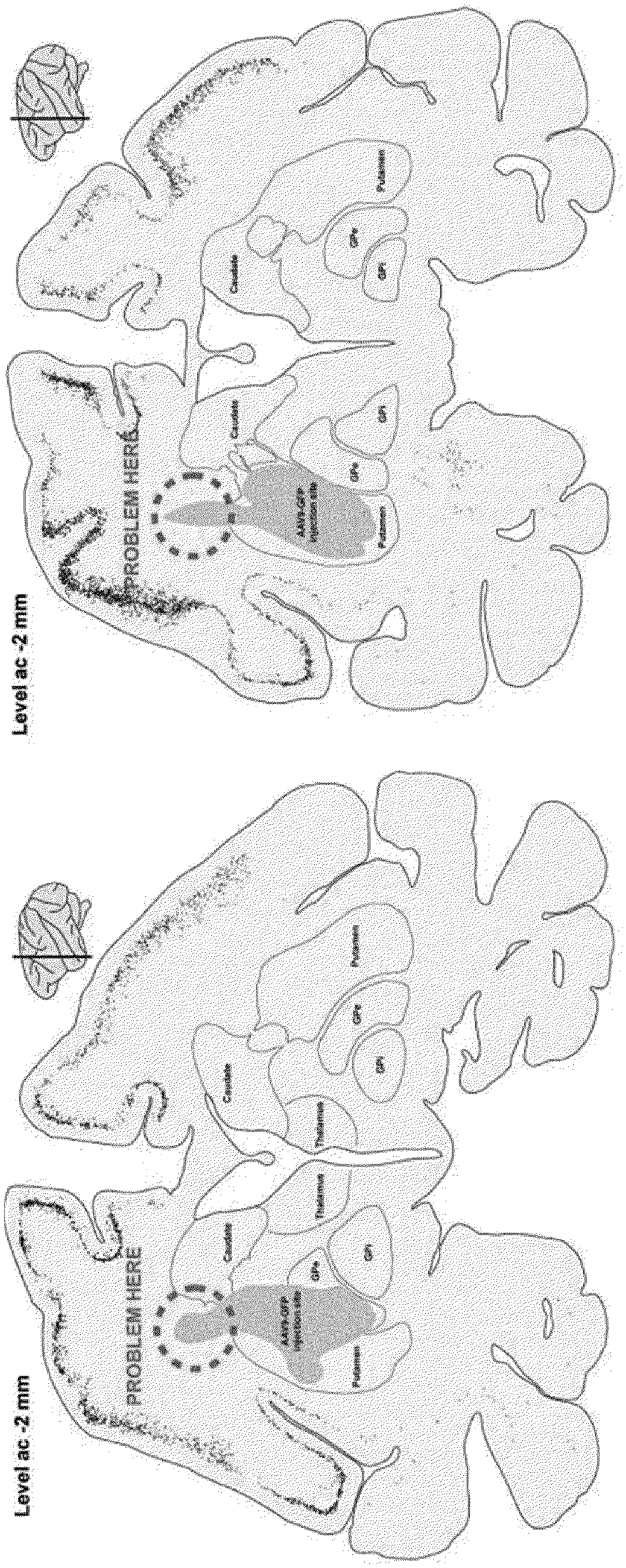

Level ac -2 mm
PROBLEM HERE
Caudate
GPe
GPi
Putamen
AAV5-GFP injection site
Thalamus

FIG. 19B

Macaque No. 295: Injection of 5 μl of AAV-TT(GFP) (1 x 10E13) into the left post-commissural putamen

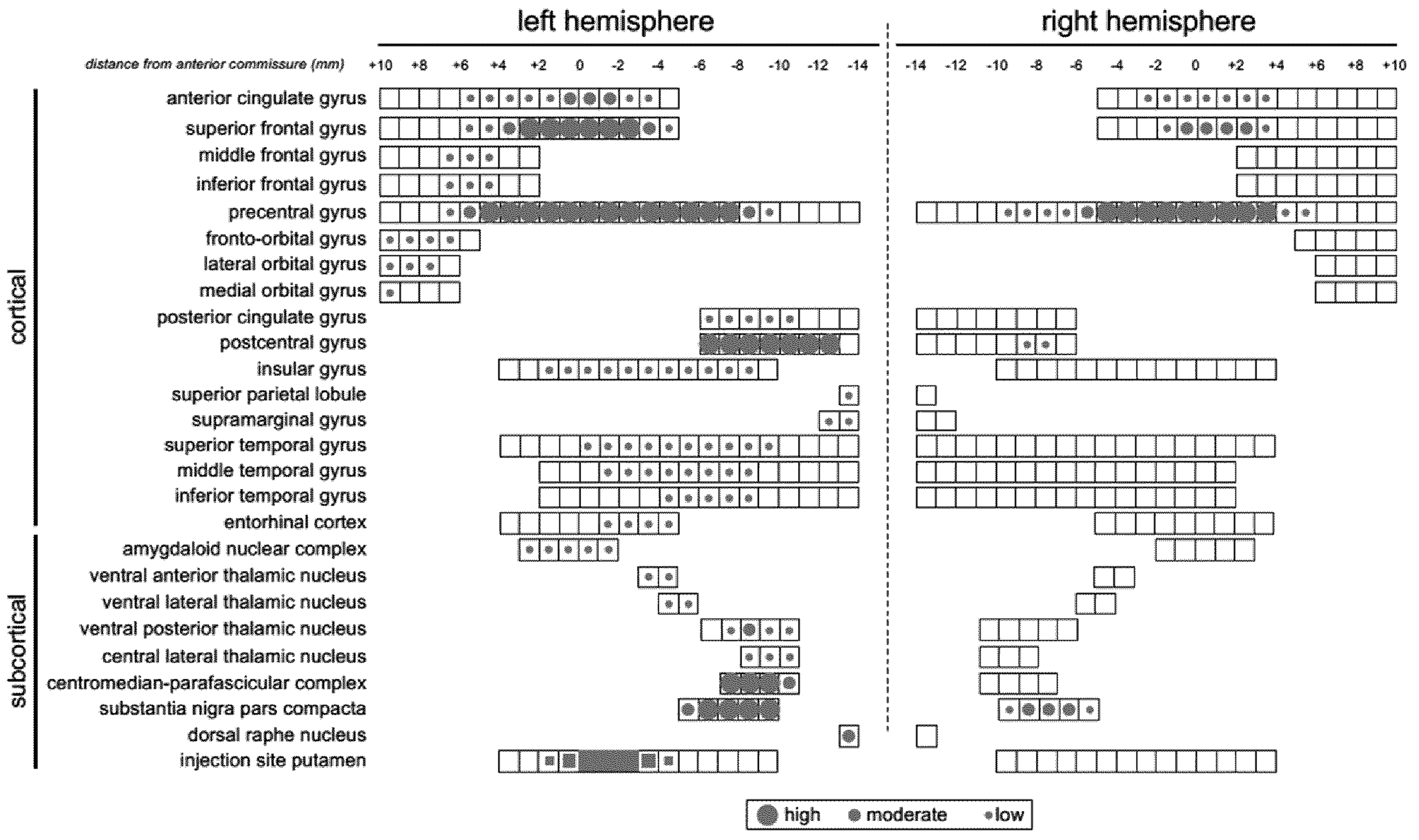


FIG. 20A

Macaque No. 296 Injection of 5 μl of AAV-TT(GFP) (1 x 10E13) into the left post-commissural putame

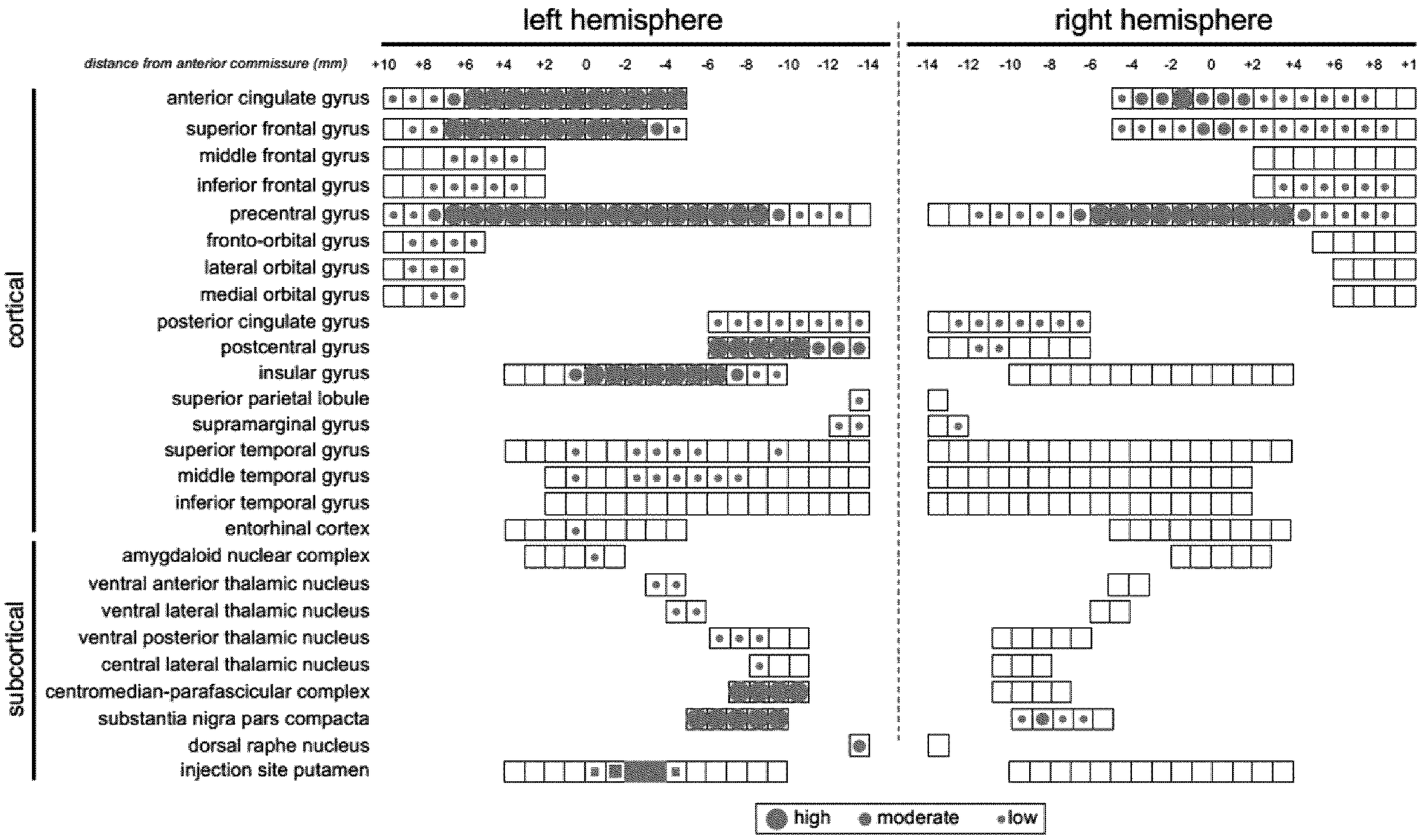


FIG. 20B

Macaque No. 297: Injection of 5 μl of AAV9(GFP) (1 x 10E13) into the left post-commissural putamen

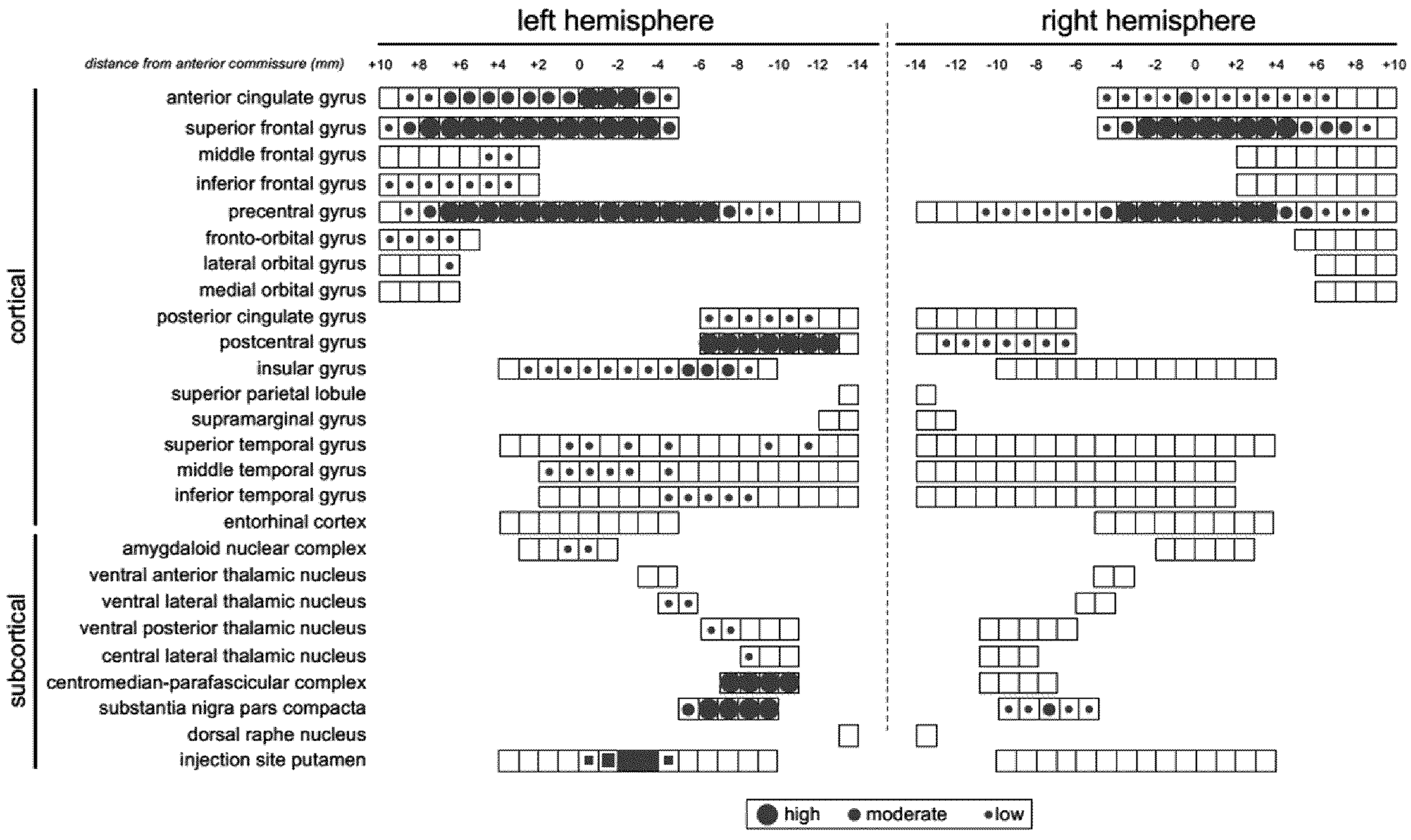


FIG. 21A

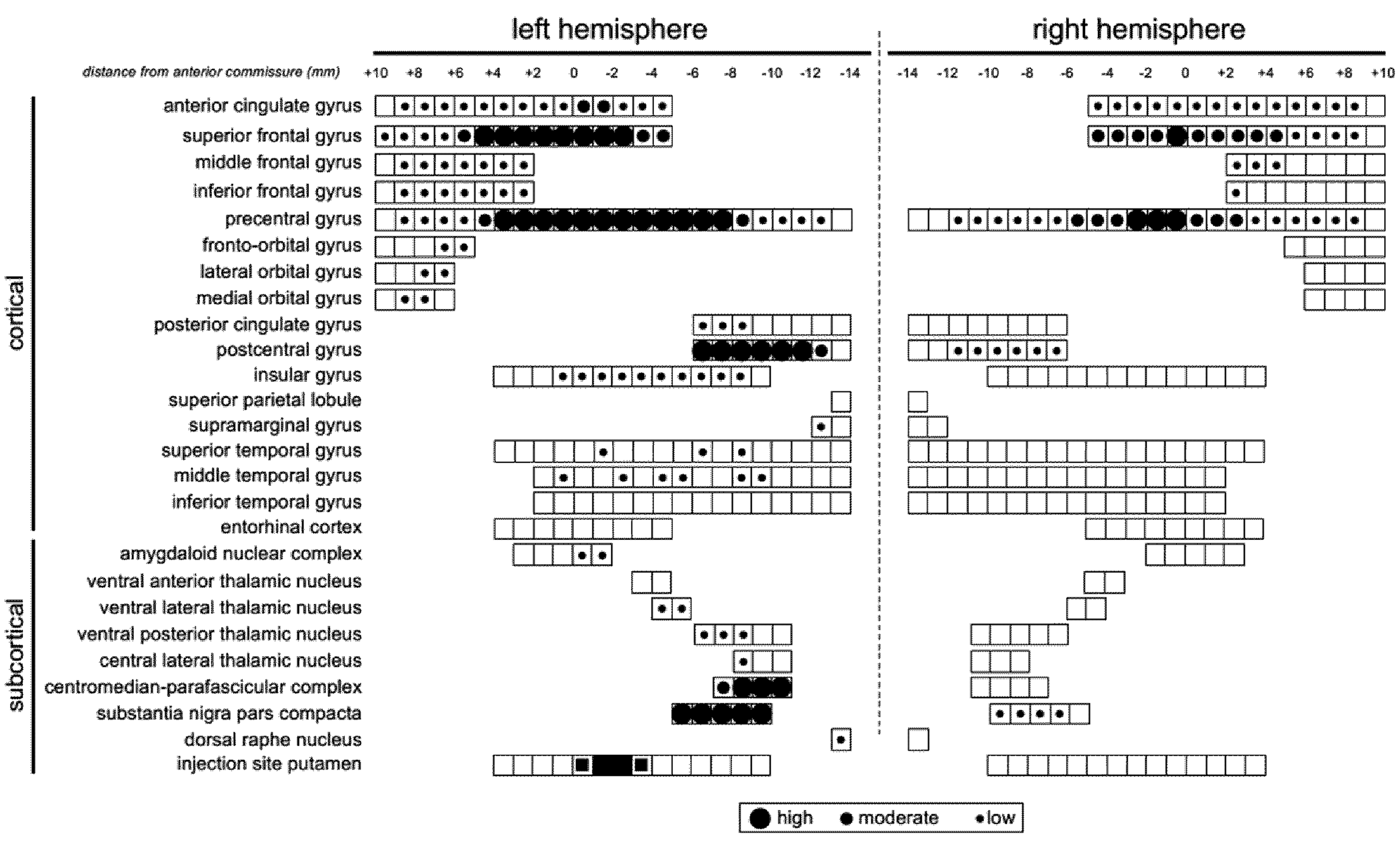
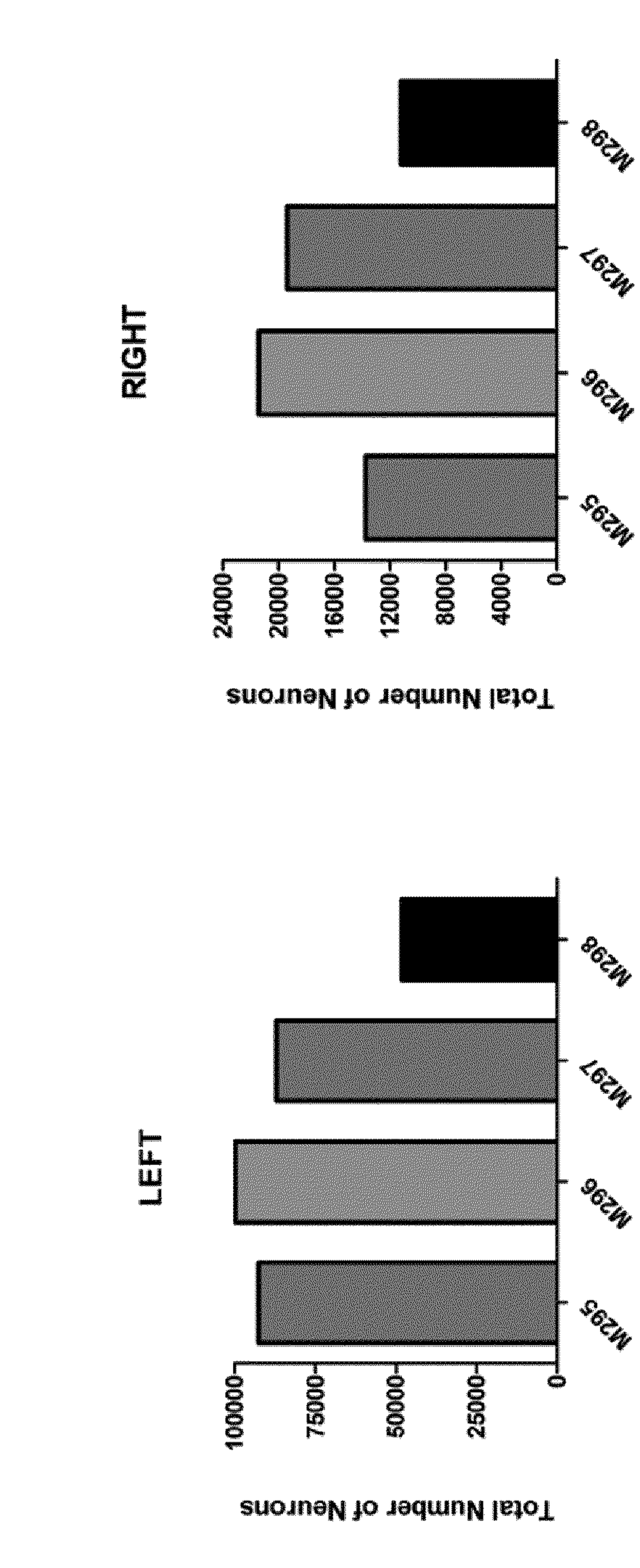

Macaque No. 298 Injection of 5 µl of AAV9(GFP) (1 x 10E13) into the left post-commissural putamen
left hemisphere
right hemisphere
distance from anterior commissure (mm)
+10 +8 +6 +4 +2 0 -2 -4 -6 -8 -10 -12 -14
-14 -12 -10 -8 -6 -4 -2 0 +2 +4 +6 +8 +10
cortical
anterior cingulate gyrus
superior frontal gyrus
middle frontal gyrus
inferior frontal gyrus
precentral gyrus
fronto-orbital gyrus
lateral orbital gyrus
medial orbital gyrus
posterior cingulate gyrus
postcentral gyrus
insular gyrus
superior parietal lobule
supramarginal gyrus
superior temporal gyrus
middle temporal gyrus
inferior temporal gyrus
entorhinal cortex
subcortical
amygdaloid nuclear complex
ventral anterior thalamic nucleus
ventral lateral thalamic nucleus
ventral posterior thalamic nucleus
central lateral thalamic nucleus
centromedian-parafascicular complex
substantia nigra pars compacta
dorsal raphe nucleus
injection site putamen
high
moderate
low

FIG. 21B


RIGHT
Total Number of Neurons
24000
20000
16000
12000
8000
4000
0
M295
M296
M297
M298
LEFT
Total Number of Neurons
100000
75000
50000
25000
0
M295
M296
M297
M298

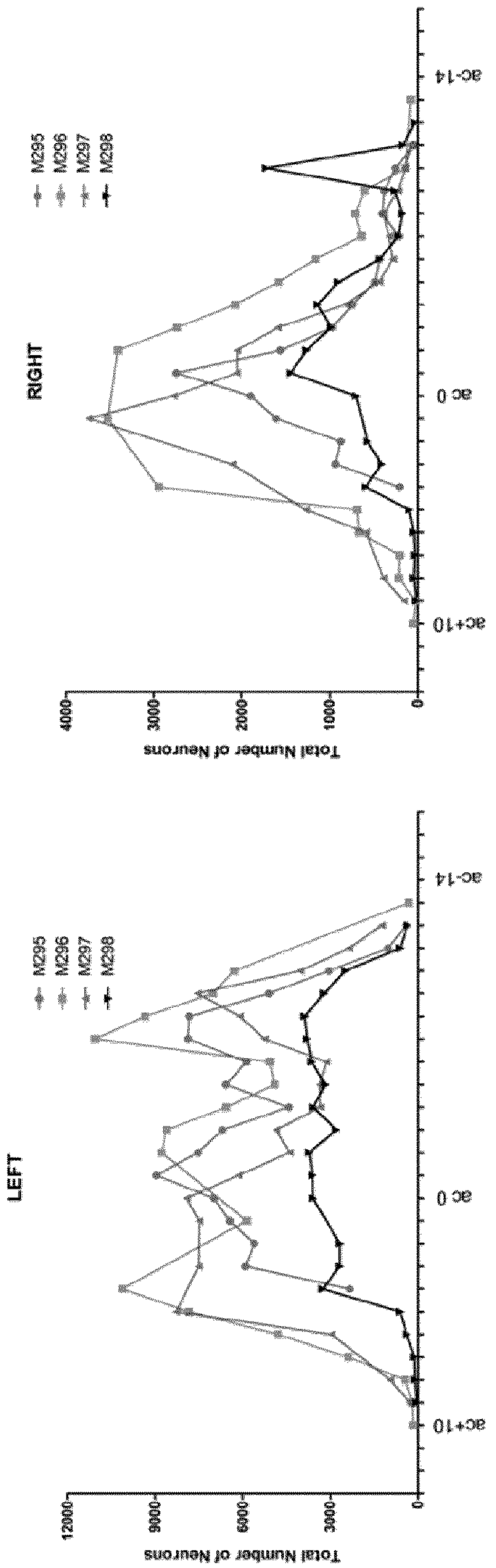
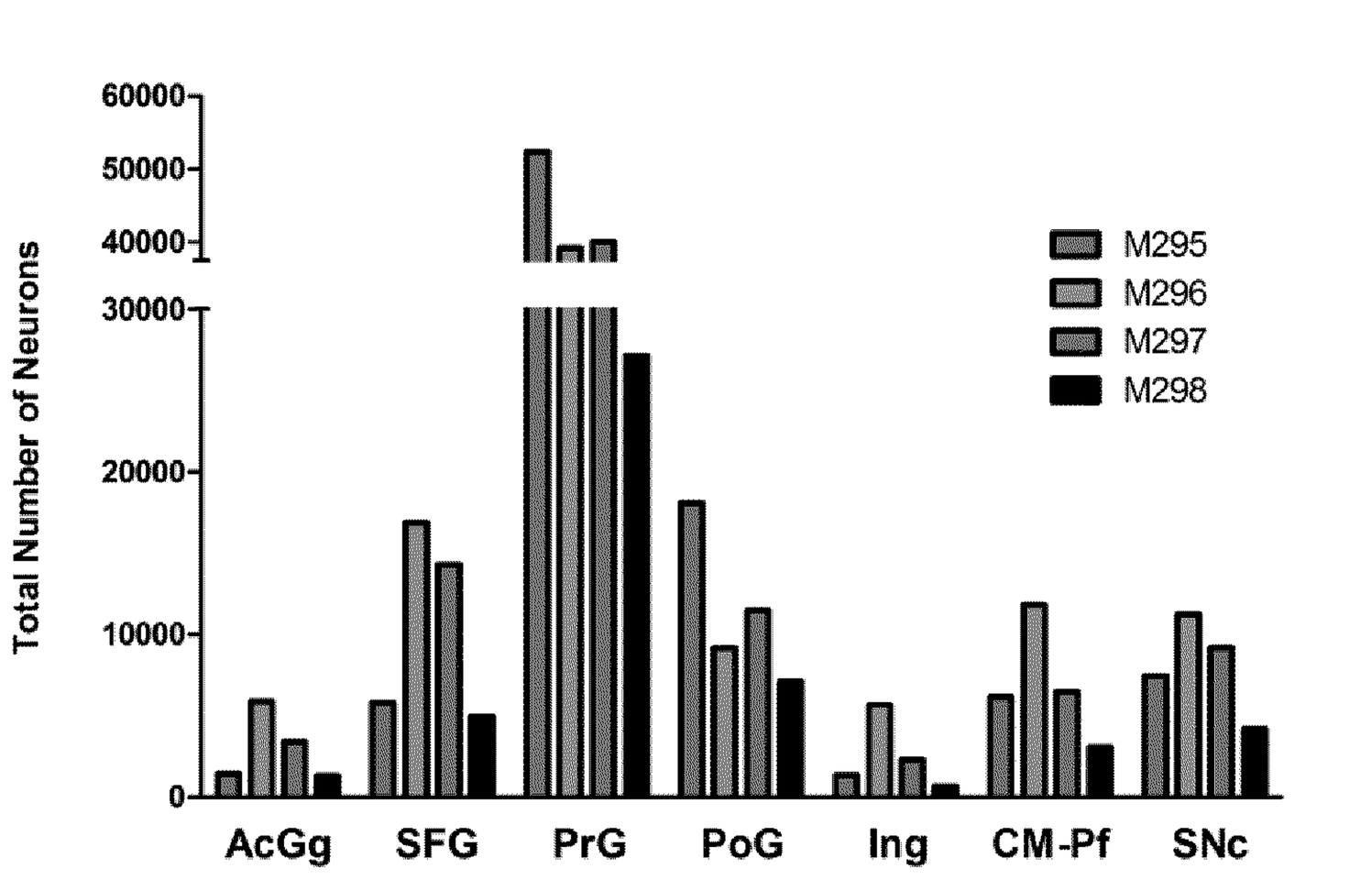

RIGHT
Total Number of Neurons
4000
3000
2000
1000
0
ac+10
ac 0
ac-14
M295
M296
M297
M298
LEFT
Total Number of Neurons
12000
9000
6000
3000
0
ac+10
ac 0
ac-14

FIG. 22


LEFT
Total Number of Neurons
60000
50000
40000
30000
20000
10000
0
AcGg
SFG
PrG
PoG
Ing
CM-Pf
SNc
M295
M296
M297
M298

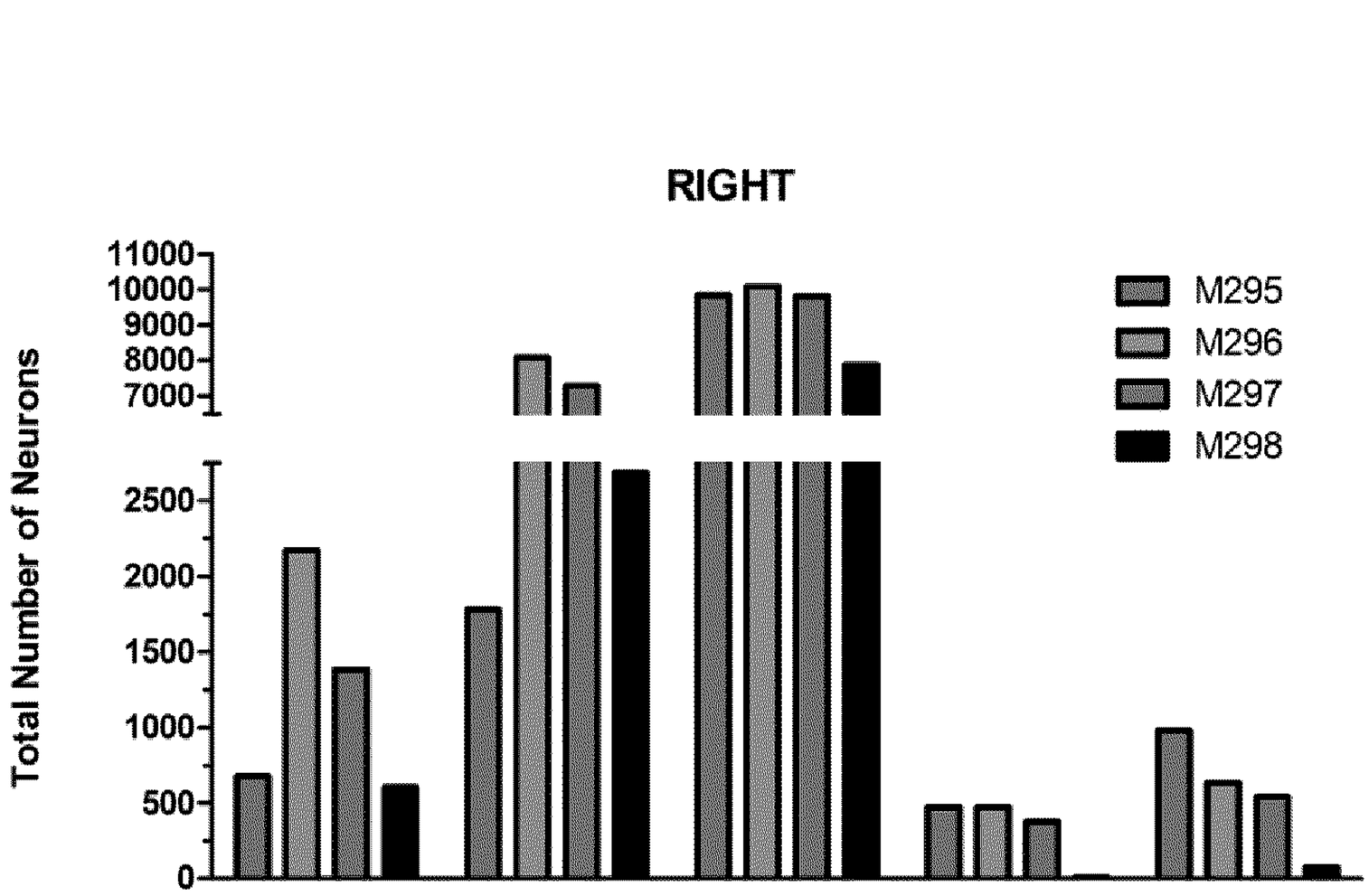

RIGHT
Total Number of Neurons
11000
10000
9000
8000
7000
2500
2000
1500
1000
500
0
AcGg
SFG
PrG
PoG
SNc
M295
M296
M297
M298

FIG. 23

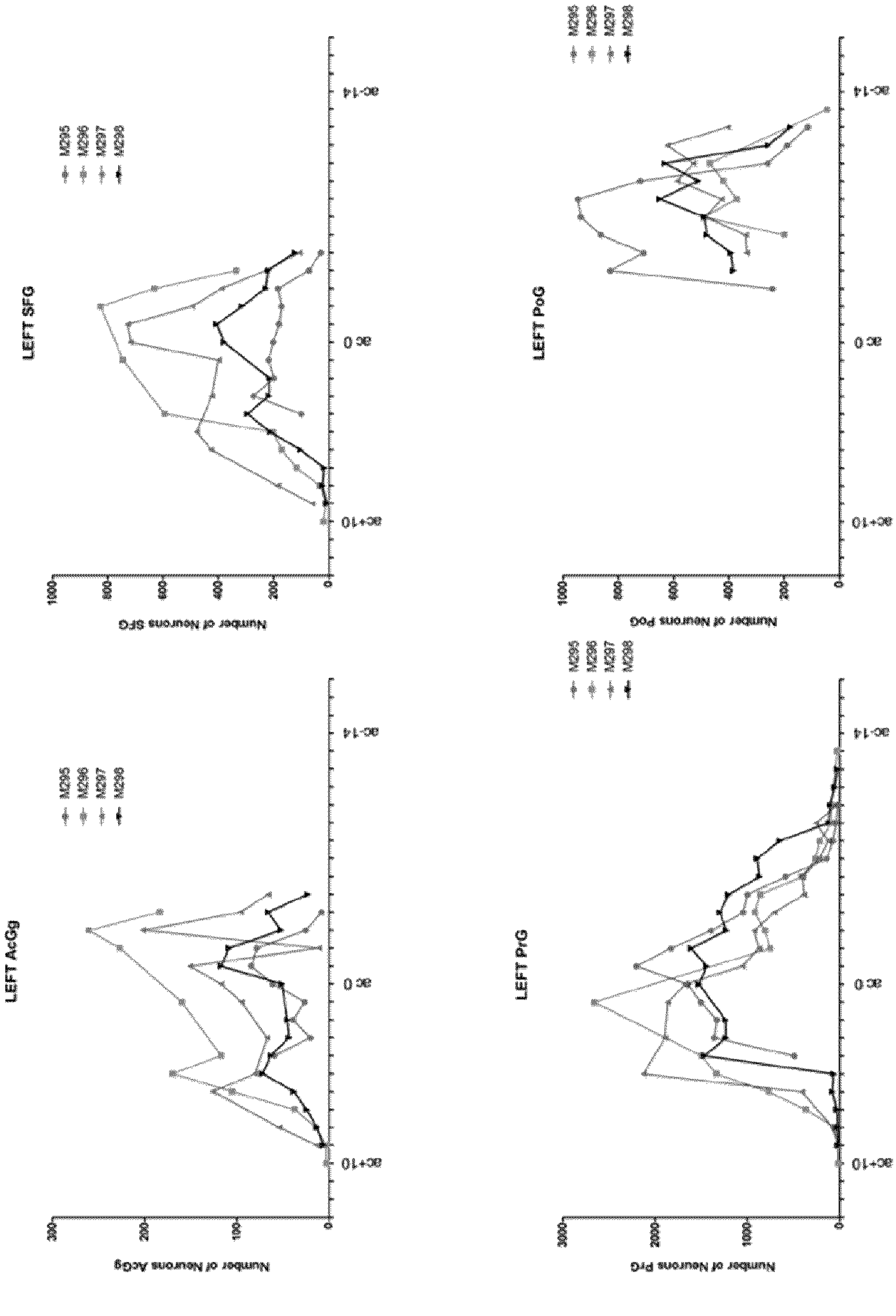
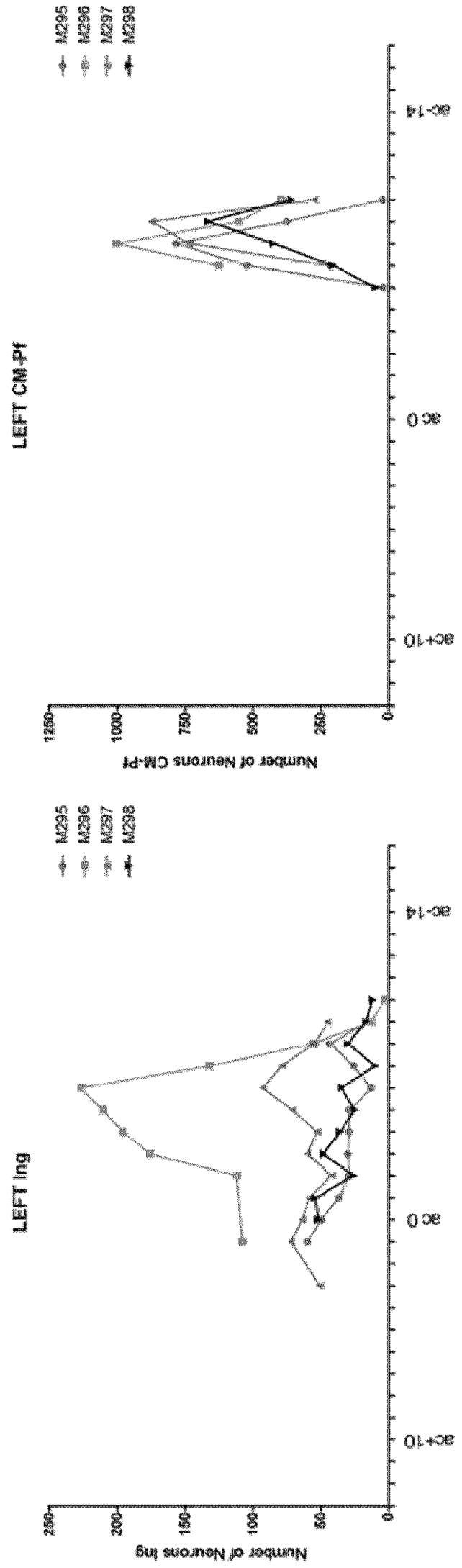

LEFT SFG
Number of Neurons SFG
LEFT PoG
Number of Neurons PoG
LEFT AcGg
Number of Neurons AcGg
LEFT PrG
Number of Neurons PrG
M295
M296
M297
M298
ac-14
ac 0
ac+10

FIG. 24



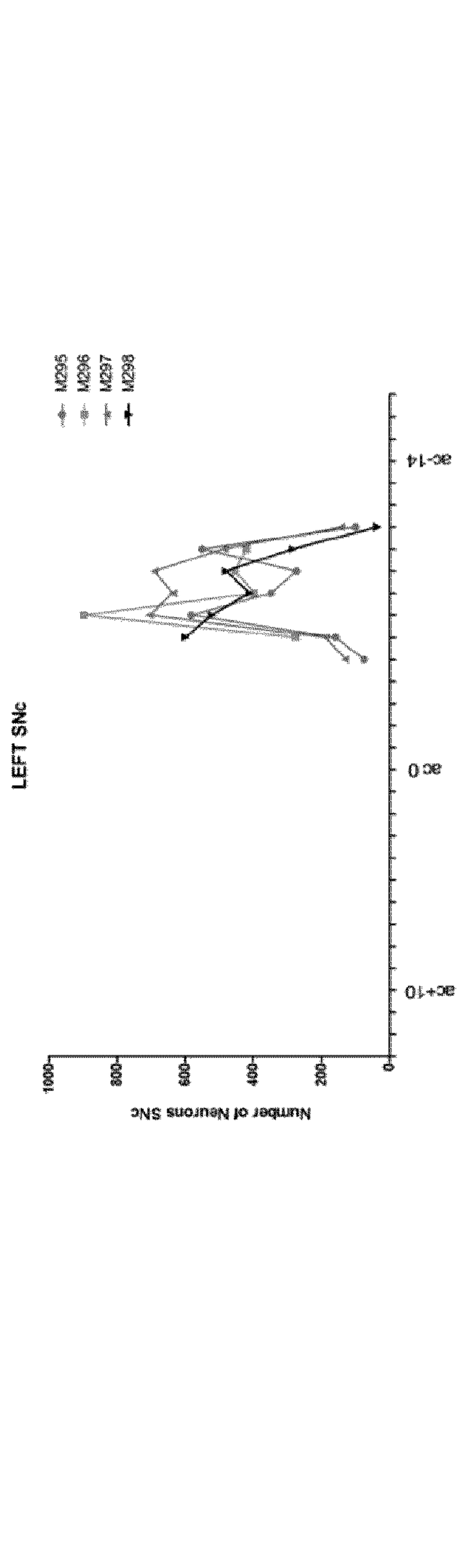


FIG.24 (CONTINUATION)

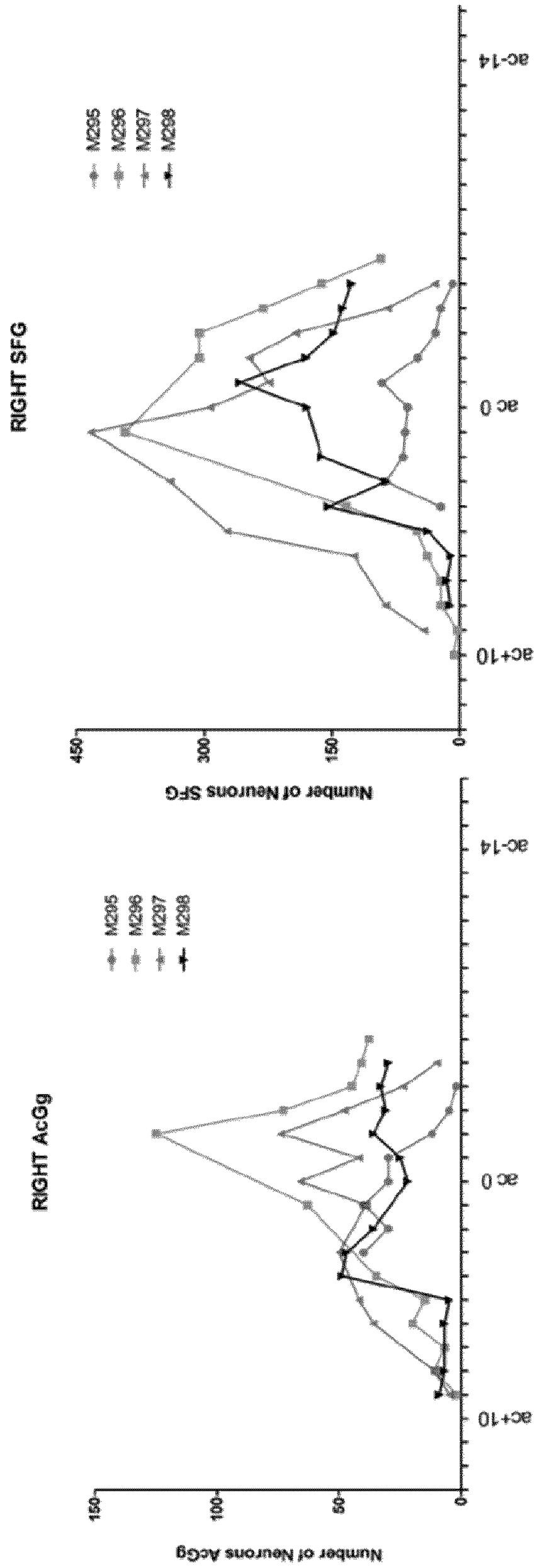

RIGHT SFG
M295
M296
M297
M298
Number of Neurons SFG
ac-14
ac 0
ac+10
0
150
300
450
RIGHT AcGg
Number of Neurons AcGg
0
50
100
150

FIG. 25

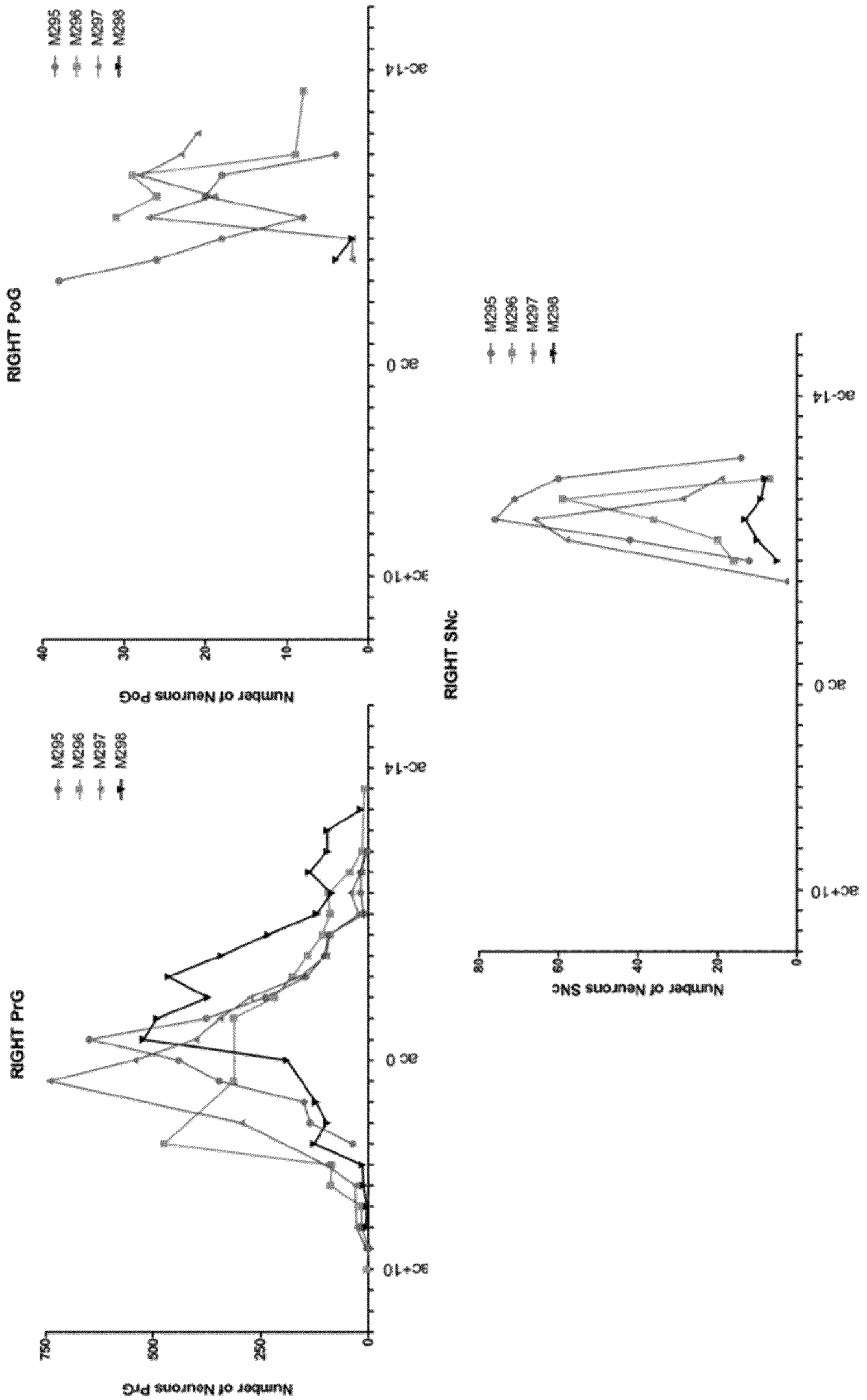


FIG. 25 (CONTINUATION)

Workplan for animals M280, M282, M283 and M287
injection of 2 x 5 µl of AAV9-SynA53T into the SNc
blood and CSF samples
injection of 2 x 10 µl of AAVtt-GBA1 into the putamen
blood and CSF samples
sacrifice
Left
Weeks
Right
1
2
3
4
5
6
7
8
9
10
11
12
microPET scan with 11C/DTBZ
microPET scan with 11C/DTBZ
injection of 2 x 5 µl of AAV9-SynA53T into the SNc
microPET scan with 11C/DTBZ
blood and CSF samples
microPET scan with 11C/DTBZ
samples of peripheral organs
Workplan for animals M281, M284, M285 and M286
injection of 2 x 5 µl of AAV9-SynA53T into the SNc
blood and CSF samples
injection of 2 x 10 µl of AAV9-GBA1 into the putamen
blood and CSF samples
sacrifice
Left
Weeks
Right
1
2
3
4
5
6
7
8
9
10
11
12
microPET scan with 11C/DTBZ
microPET scan with 11C/DTBZ
injection of 2 x 5 µl of AAV9-SynA53T into the SNc
microPET scan with 11C/DTBZ
blood and CSF samples
microPET scan with 11C/DTBZ
samples of peripheral organs

FIG. 26

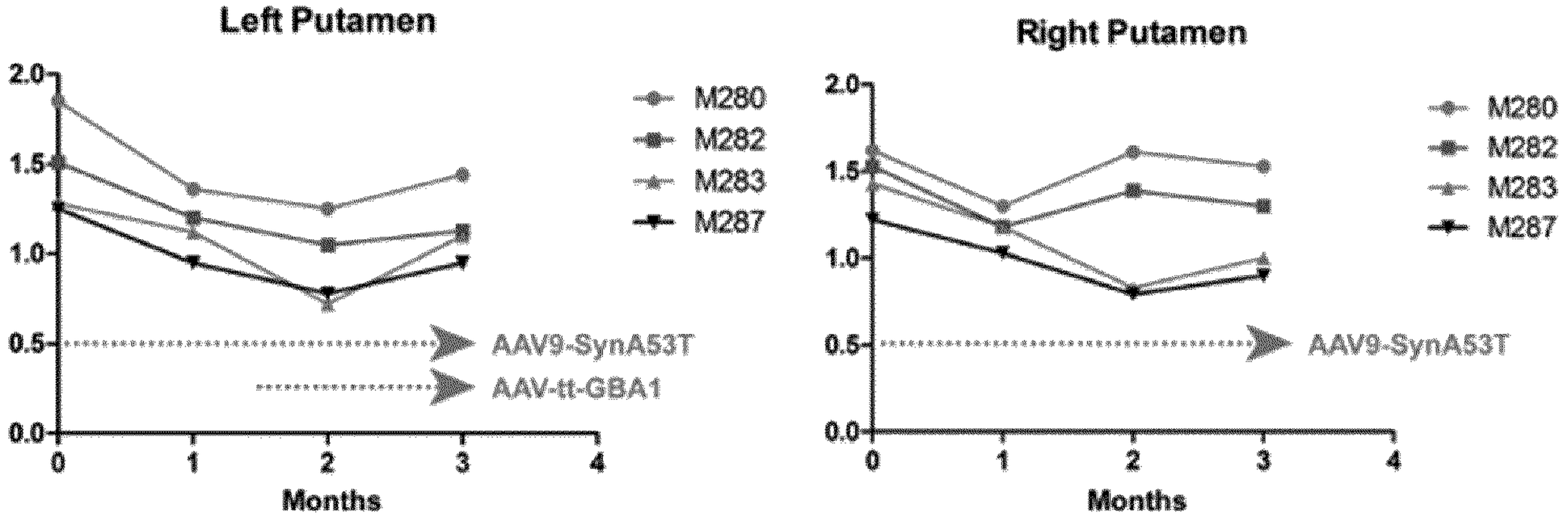

Left Putamen
Right Putamen
M280
M282
M283
M287
AAV9-SynA53T
AAV-tt-GBA1
Months
Left Putamen
Right Putamen
M281
M284
M285
M286
AAV9-SynA53T
AAV9-GBA1
Months

FIG. 27

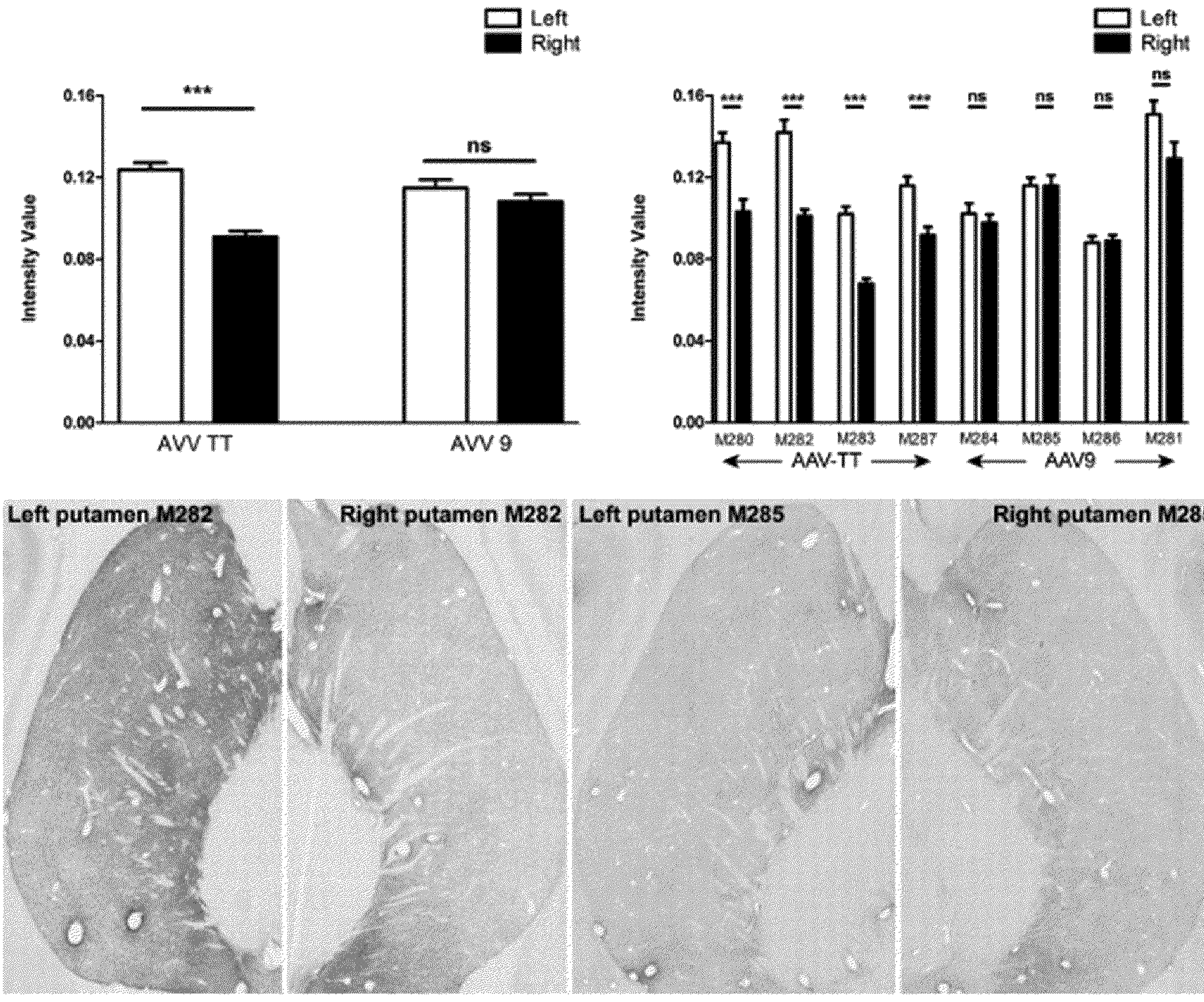

Left
Right
***
ns
0.16
0.12
0.08
0.04
0.00
Intensity Value
AVV TT
AVV 9
Left putamen M282
Right putamen M282
Left putamen M285
Right putamen M285
M280
M282
M283
M287
M284
M285
M286
M281
AAV-TT
AAV9

FIG. 28

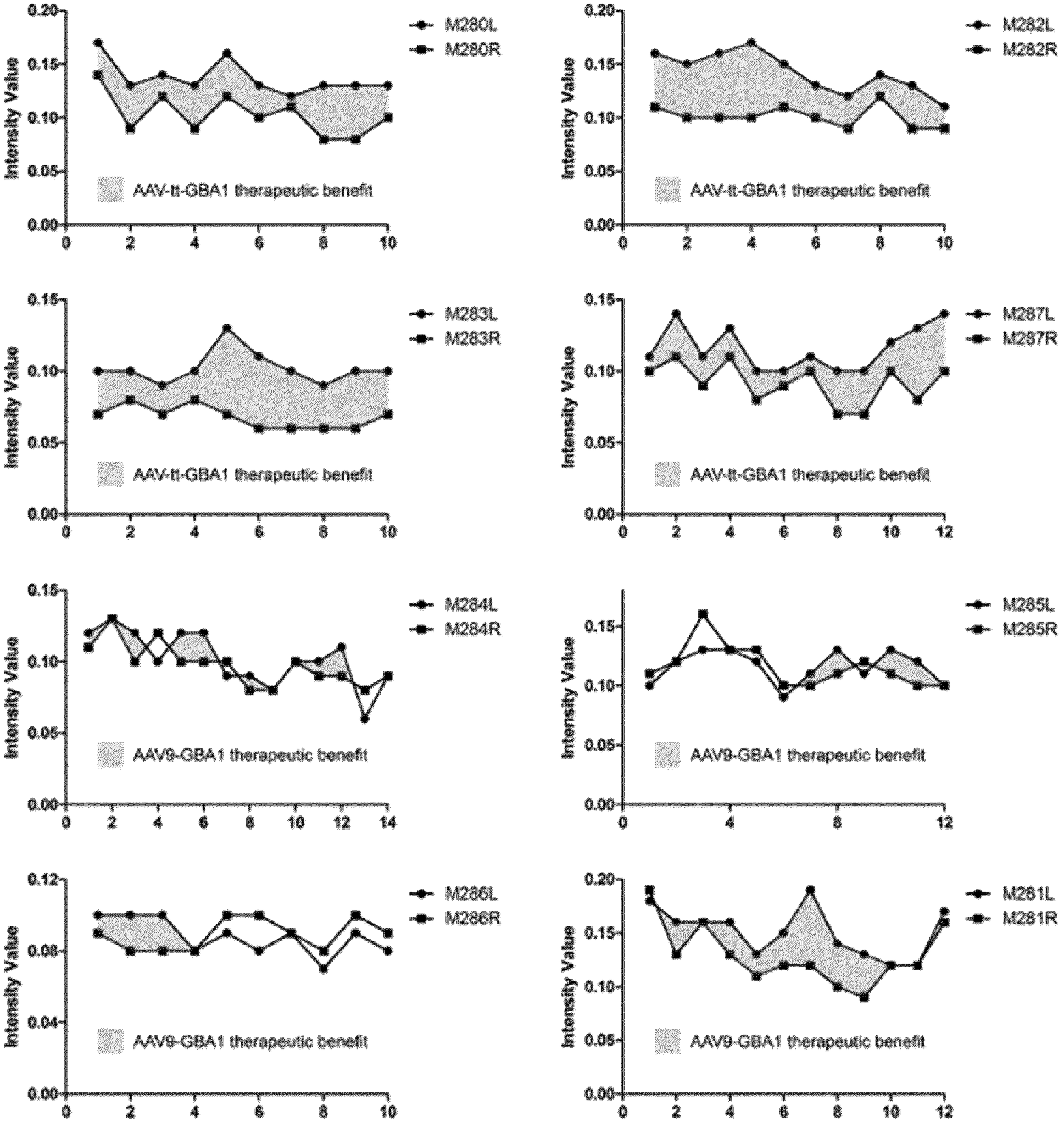
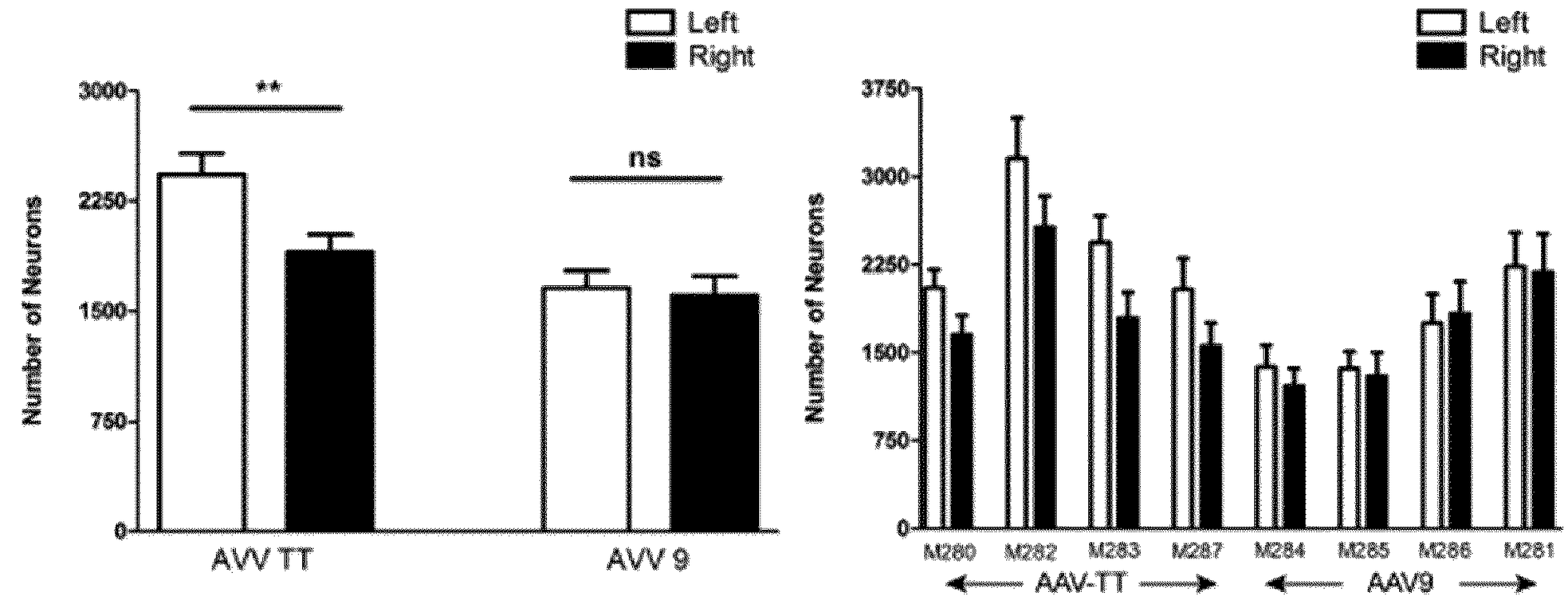

Intensity Value
AAV-tt-GBA1 therapeutic benefit
M280L
M280R
M282L
M282R
M283L
M283R
M287L
M287R
M284L
M284R
M285L
M285R
AAV9-GBA1 therapeutic benefit
M286L
M286R
M281L
M281R

FIG. 29


Left
Right
**
ns
Number of Neurons
3000
2250
1500
750
0
AVV TT
AVV 9
Left
Right
Number of Neurons
3750
3000
2250
1500
750
0
M280
M282
M283
M287
M284
M285
M286
M281
AAV-TT
AAV9

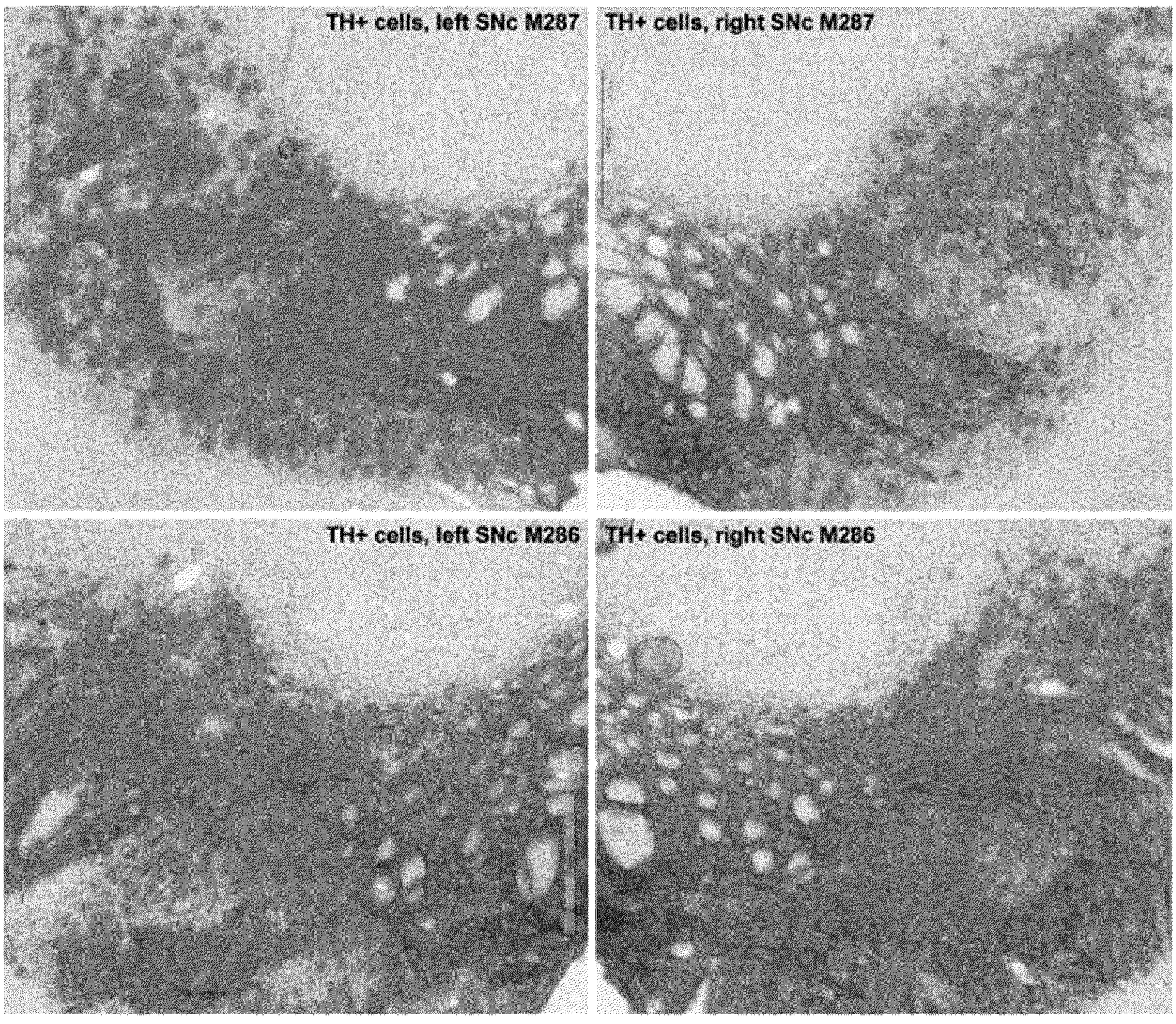

TH+ cells, left SNc M287
TH+ cells, right SNc M287
TH+ cells, left SNc M286
TH+ cells, right SNc M286

FIG. 30

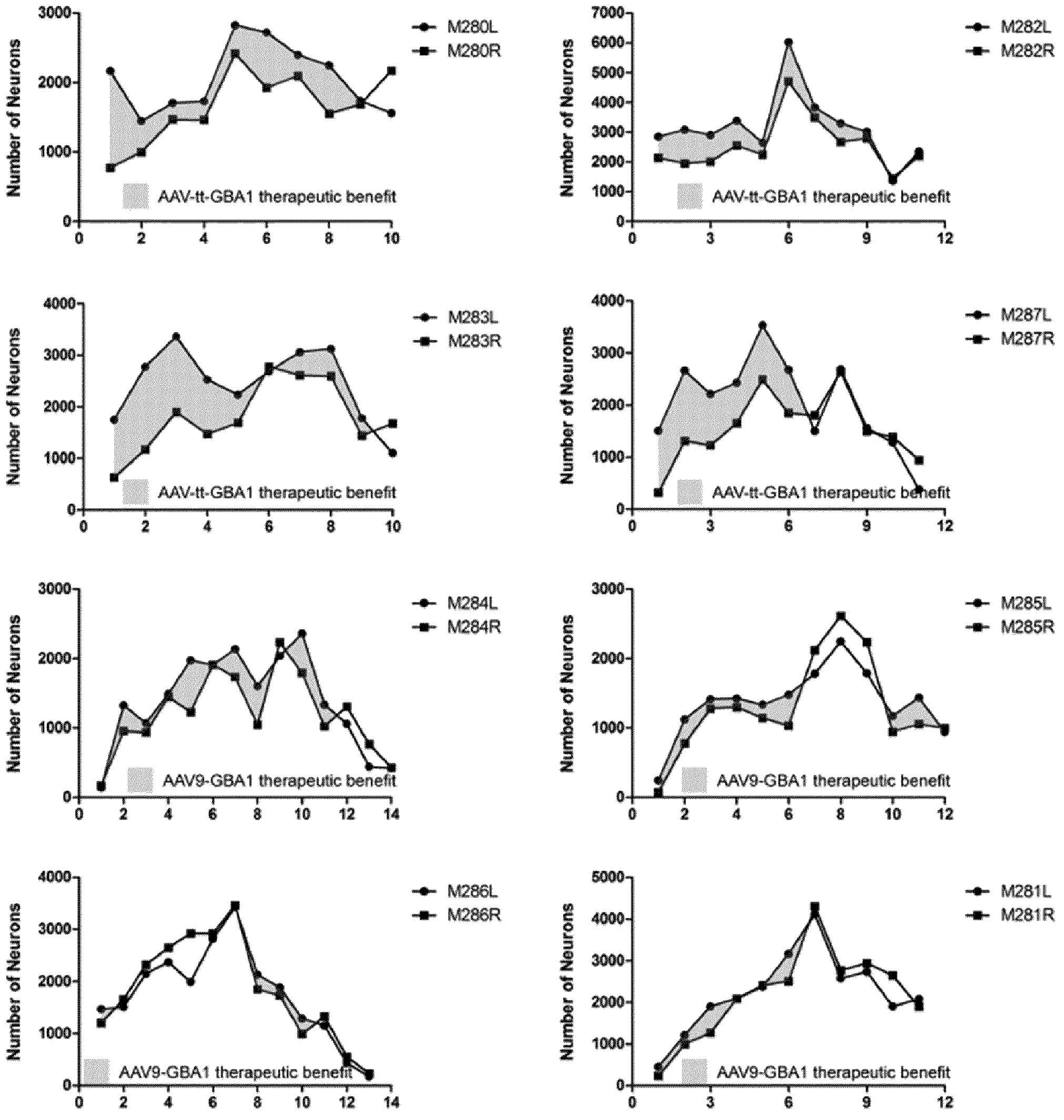

Number of Neurons
AAV-tt-GBA1 therapeutic benefit
M280L
M280R
M282L
M282R
M283L
M283R
M287L
M287R
AAV9-GBA1 therapeutic benefit
M284L
M284R
M285L
M285R
M286L
M286R
M281L
M281R

FIG. 31

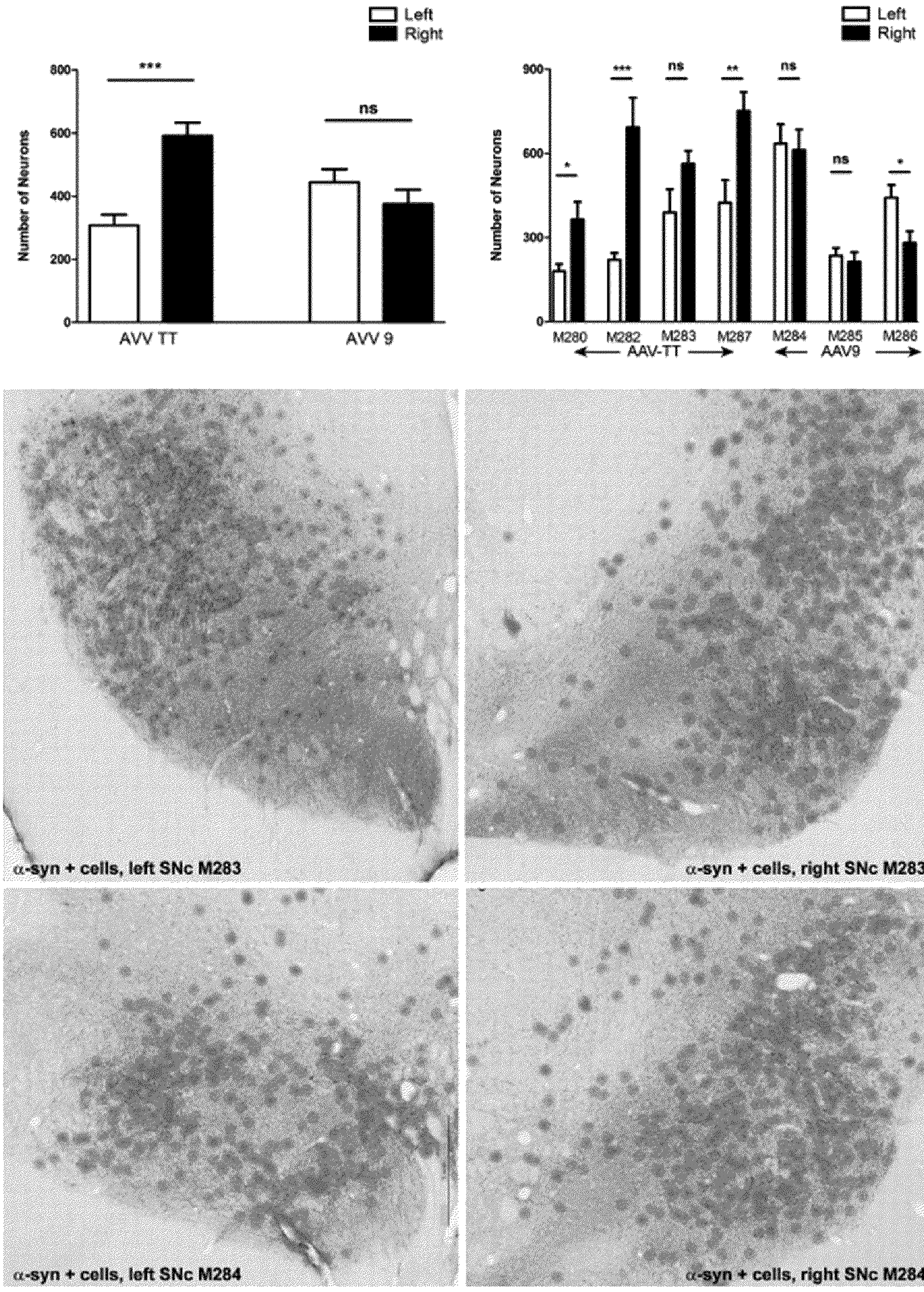
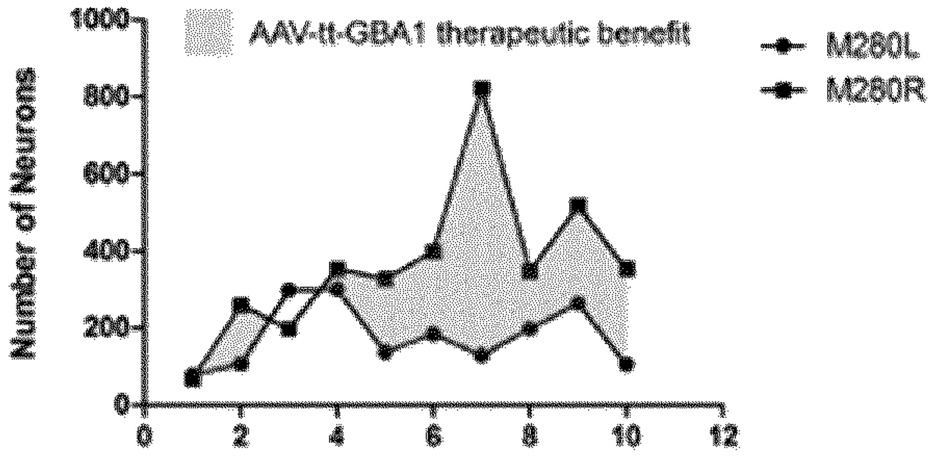
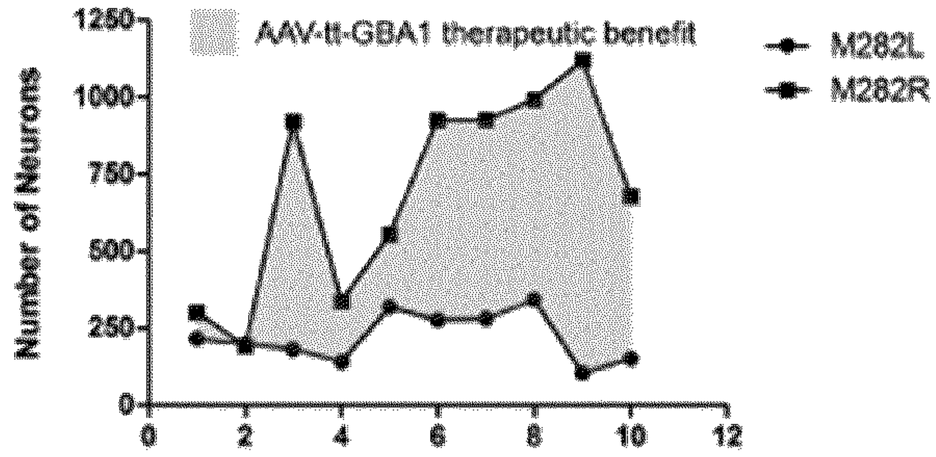
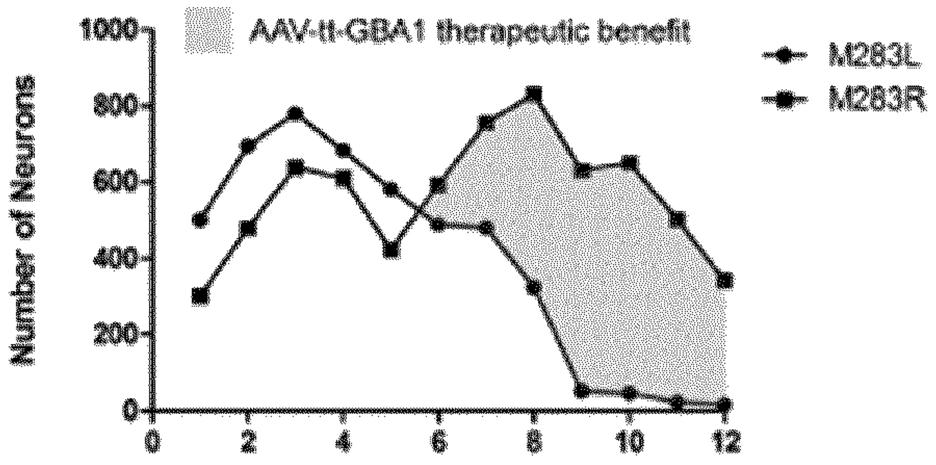
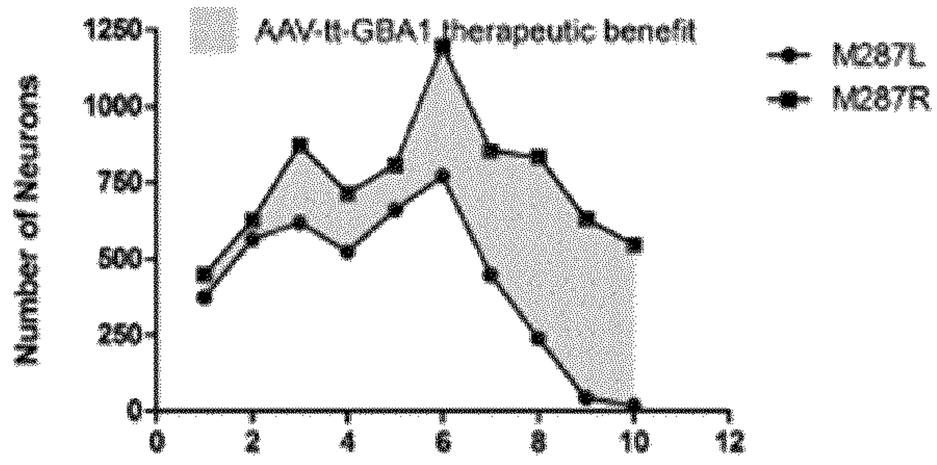

Left
Right
Number of Neurons
800
600
400
200
0
***
ns
AVV TT
AVV 9
Left
Right
Number of Neurons
900
600
300
0
***
ns
**
ns
*
ns
*
M280
M282
M283
M287
M284
M285
M286
AAV-TT
AAV9
α-syn + cells, left SNc M283
α-syn + cells, right SNc M283
α-syn + cells, left SNc M284
α-syn + cells, right SNc M284

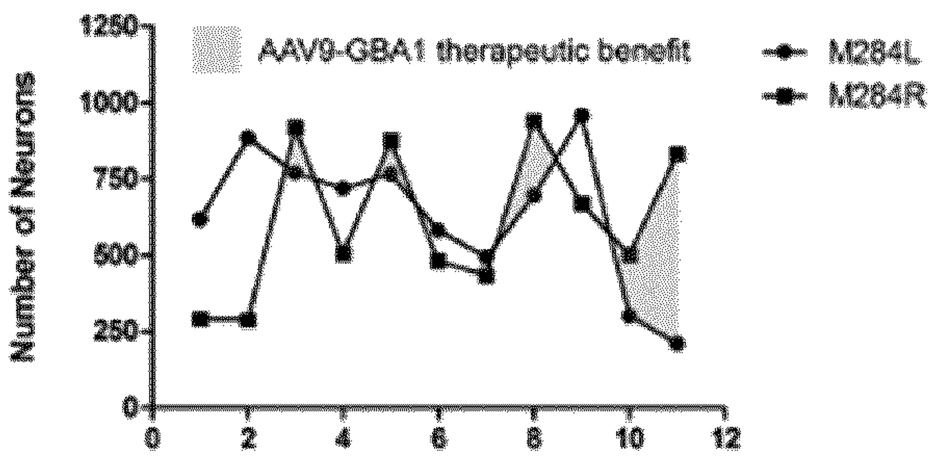
FIG. 32

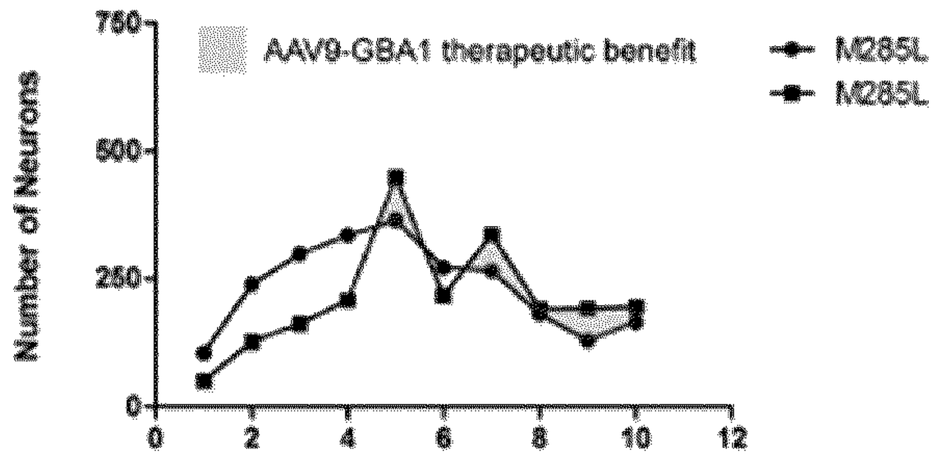

AAV-tt-GBA1 therapeutic benefit
M280L
M280R
Number of Neurons
0
200
400
600
800
1000
0
2
4
6
8
10
12


AAV-tt-GBA1 therapeutic benefit
M282L
M282R
Number of Neurons
0
250
500
750
1000
1250
0
2
4
6
8
10
12


AAV-tt-GBA1 therapeutic benefit
M283L
M283R
Number of Neurons
0
200
400
600
800
1000
0
2
4
6
8
10
12


AAV-tt-GBA1 therapeutic benefit
M287L
M287R
Number of Neurons
0
250
500
750
1000
1250
0
2
4
6
8
10
12


AAV9-GBA1 therapeutic benefit
M284L
M284R
Number of Neurons
0
250
500
750
1000
1250
0
2
4
6
8
10
12


AAV9-GBA1 therapeutic benefit
M285L
M285L
Number of Neurons
0
250
500
750
0
2
4
6
8
10
12

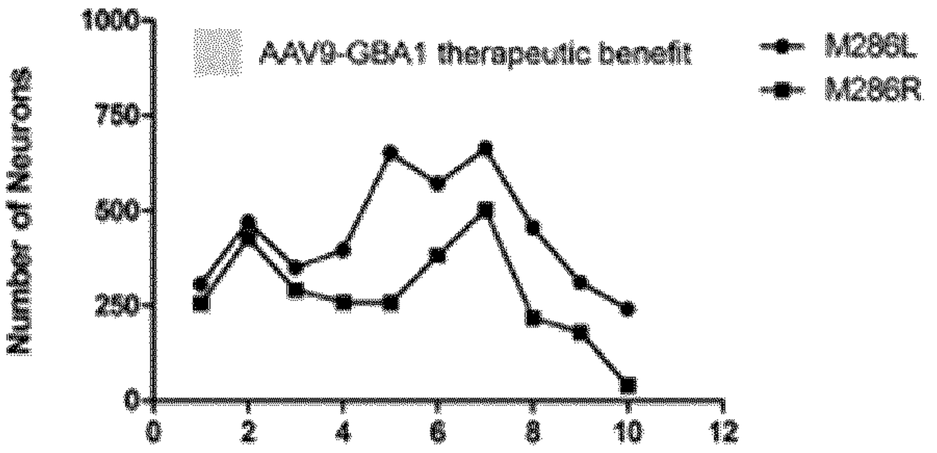

AAV9-GBA1 therapeutic benefit
M286L
M286R
Number of Neurons
0
250
500
750
1000
0
2
4
6
8
10
12

FIG. 33

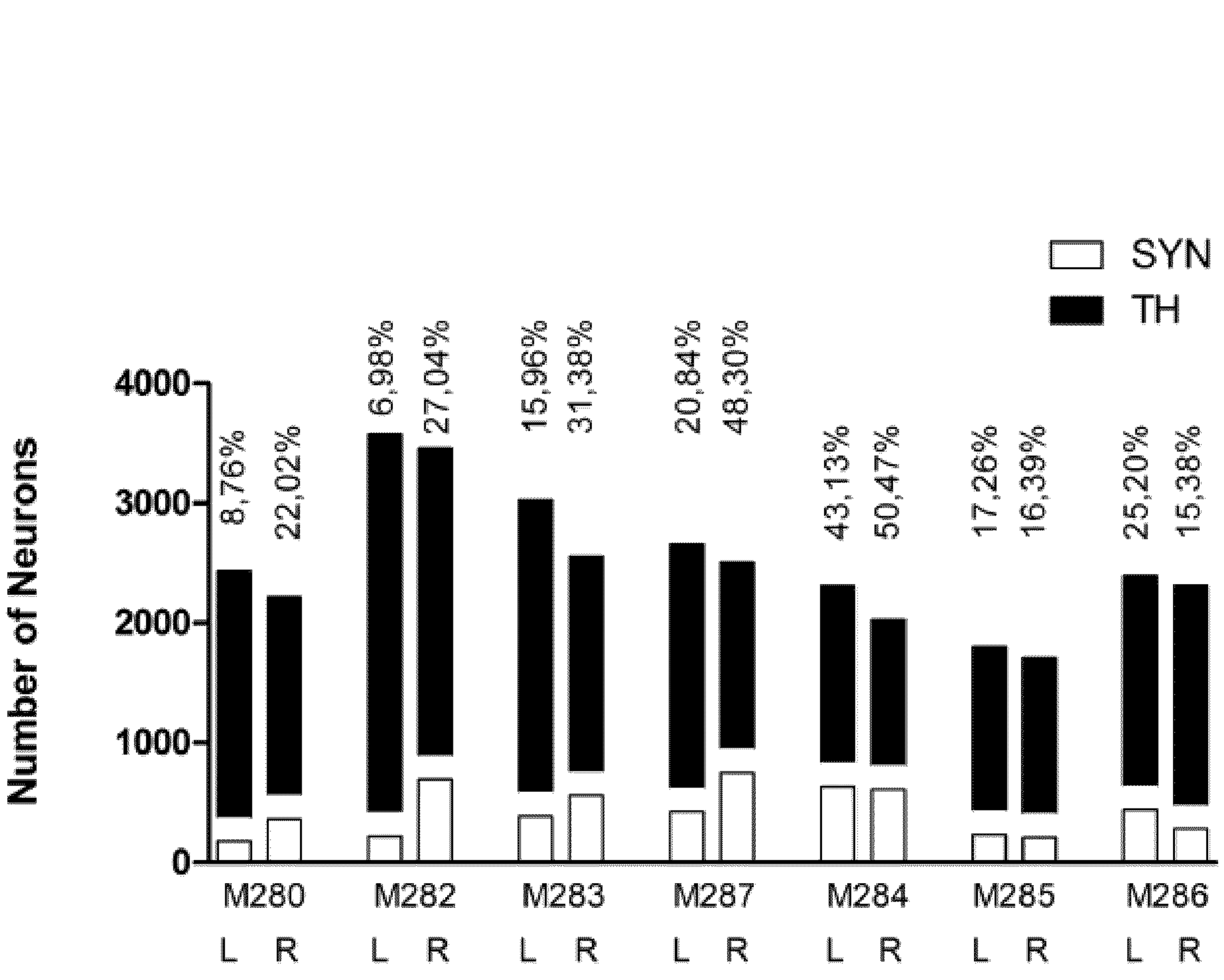

Number of Neurons
4000
3000
2000
1000
0
SYN
TH
8,76%
22,02%
6,98%
27,04%
15,96%
31,38%
20,84%
48,30%
43,13%
50,47%
17,26%
16,39%
25,20%
15,38%
M280
M282
M283
M287
M284
M285
M286
L R L R L R L R L R L R L R

FIG. 34