Figure 1

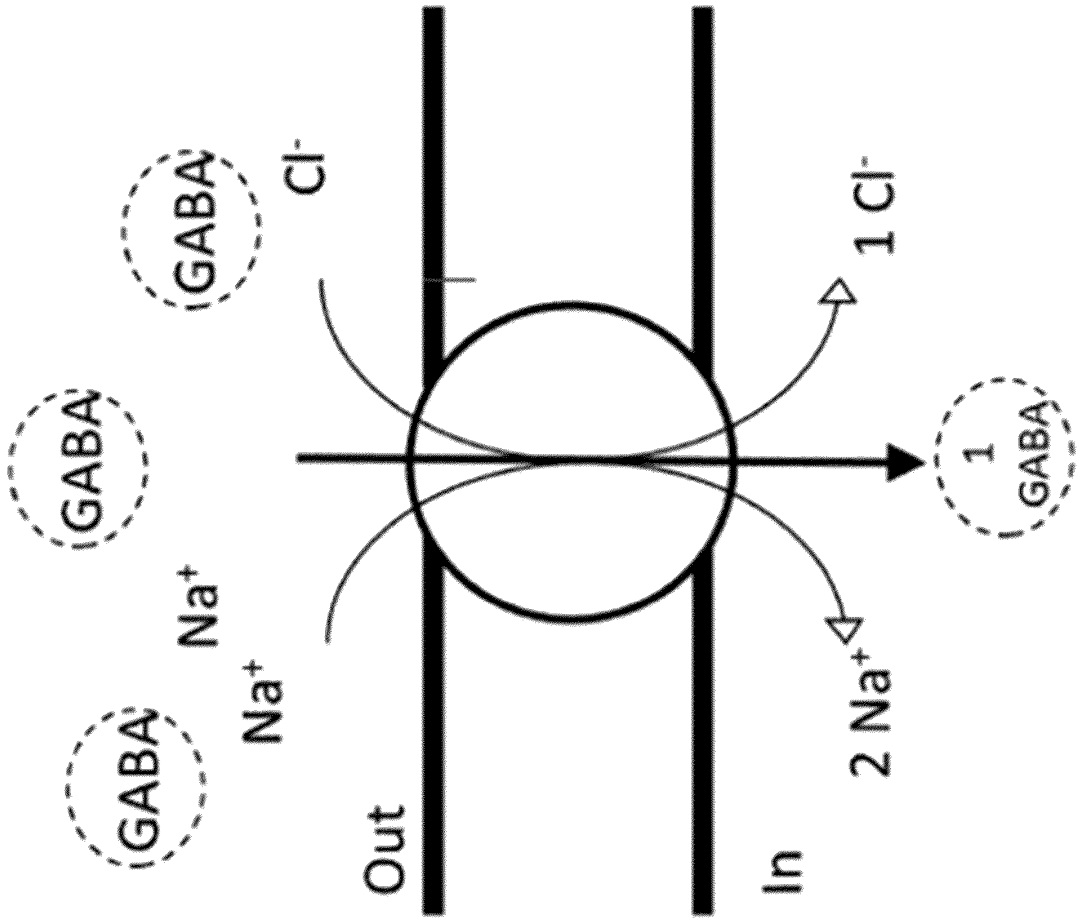


Figure 2

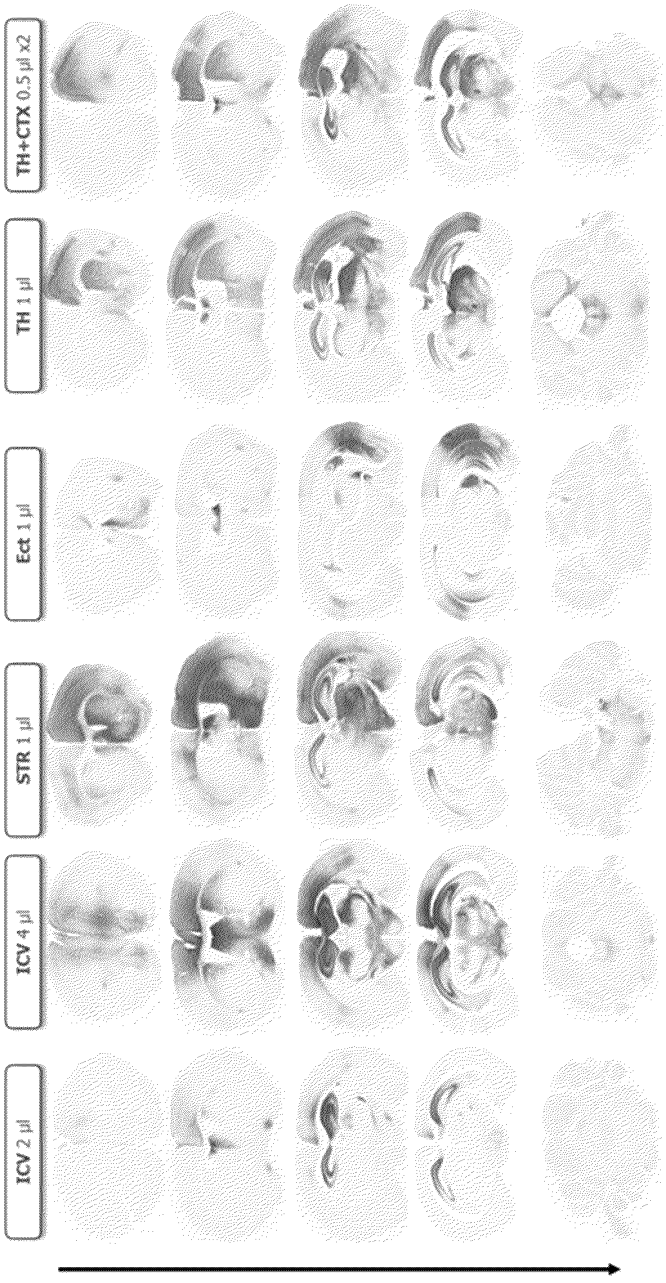


Figure 3

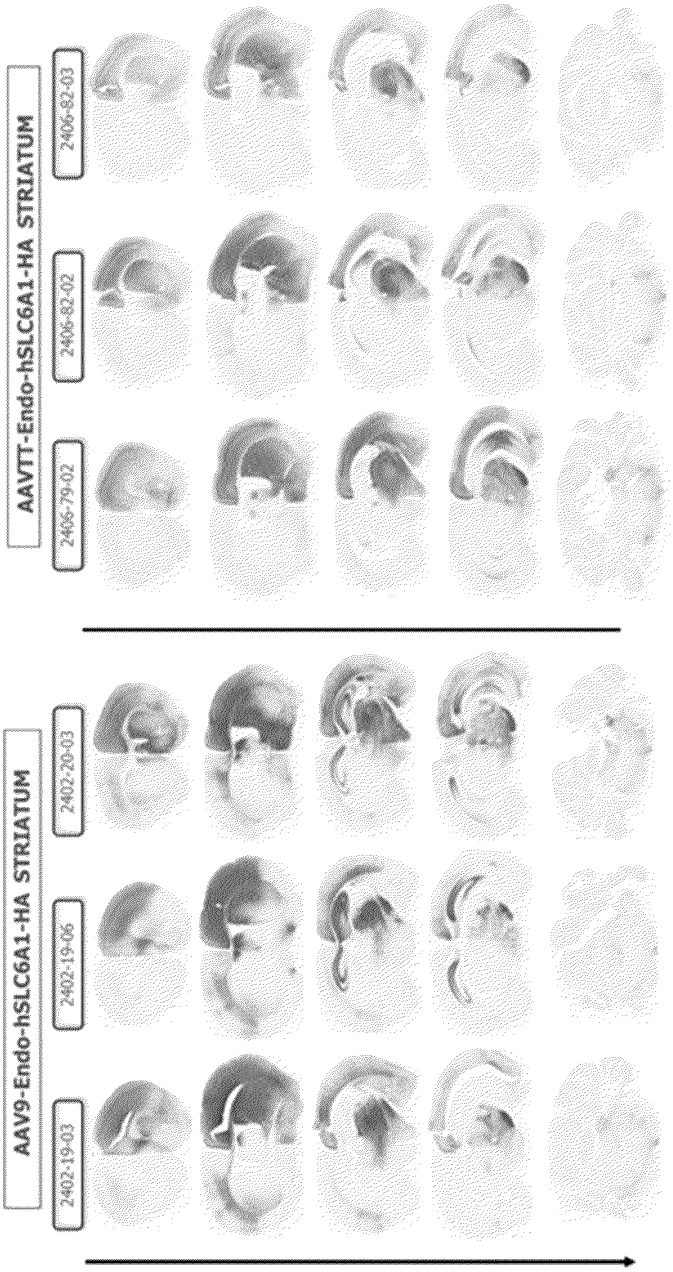

AAVTT-Endo-hSLC6A1-HA STRIATUM
2406-82-03
2406-82-02
2406-79-02
AAV9-Endo-hSLC6A1-HA STRIATUM
2402-20-03
2402-19-06
2402-19-03

Figure 4

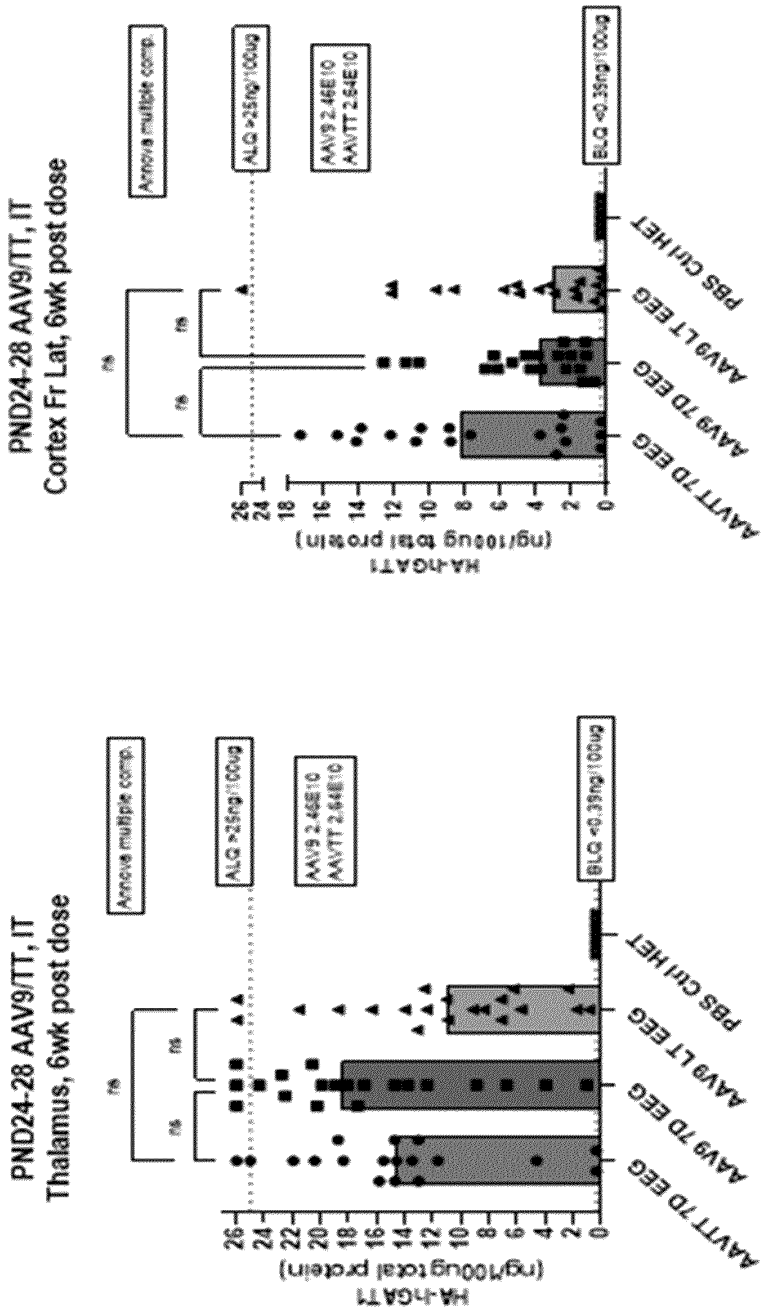

PND24-28 AAV9/TT, IT
Cortex Fr Lat, 6wk post dose
Anova multiple comp.
ALQ >25ng/100ug
AAV9 2.46E10
AAVTT 2.64E10
BLQ <0.39ng/100ug
ns
ns
ns
26
24
18
16
14
12
10
8
6
4
2
0
hA-hGAT1
(ng/100ug total protein)
AAVTT 7D EEG
AAV9 7D EEG
AAV9 LT EEG
PBS Ctrl HET
PND24-28 AAV9/TT, IT
Thalamus, 6wk post dose
Anova multiple comp.
ALQ >25ng/100ug
AAV9 2.46E10
AAVTT 2.64E10
BLQ <0.39ng/100ug
ns
ns
ns
26
24
22
20
18
16
14
12
10
8
6
4
2
0
hA-hGAT1
(ng/100ug total protein)
AAVTT 7D EEG
AAV9 7D EEG
AAV9 LT EEG
PBS Ctrl HET

Figure 4 continued

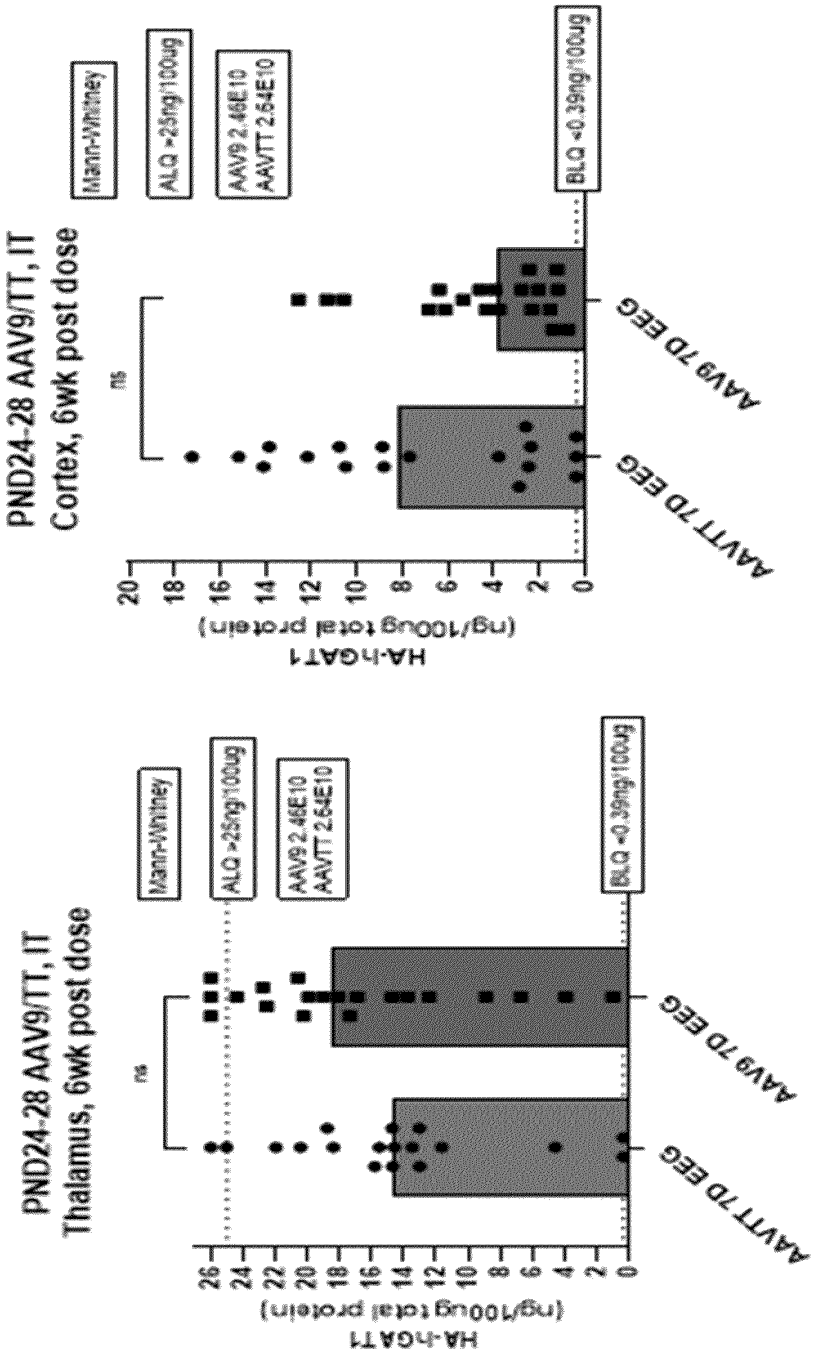
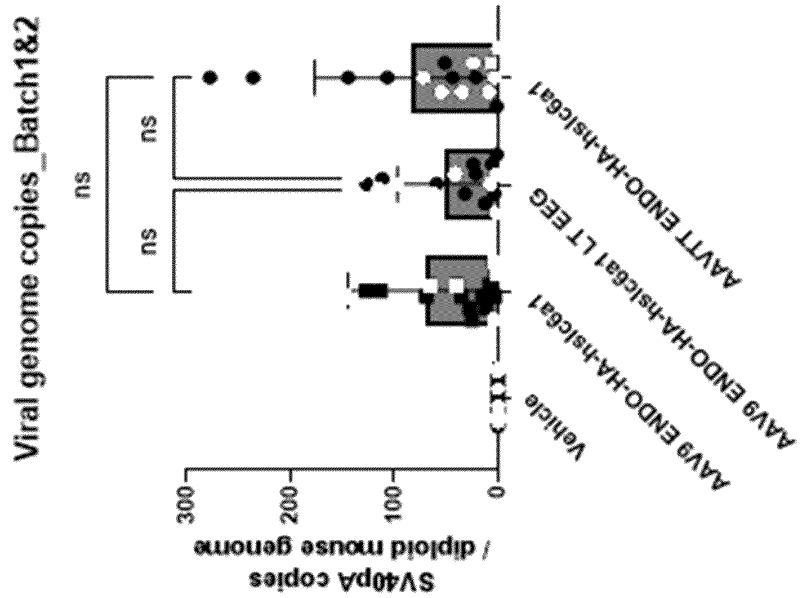


Figure 5

(A)


Viral genome copies_Batch1&2
ns
ns
ns
SV40pA copies / diploid mouse genome
300
200
100
0
Vehicle
AAV9 ENDO-HA-hslc6a1
AAV9 ENDO-HA-hslc6a1 LT EEG
AAVTT ENDO-HA-hslc6a1

(B)

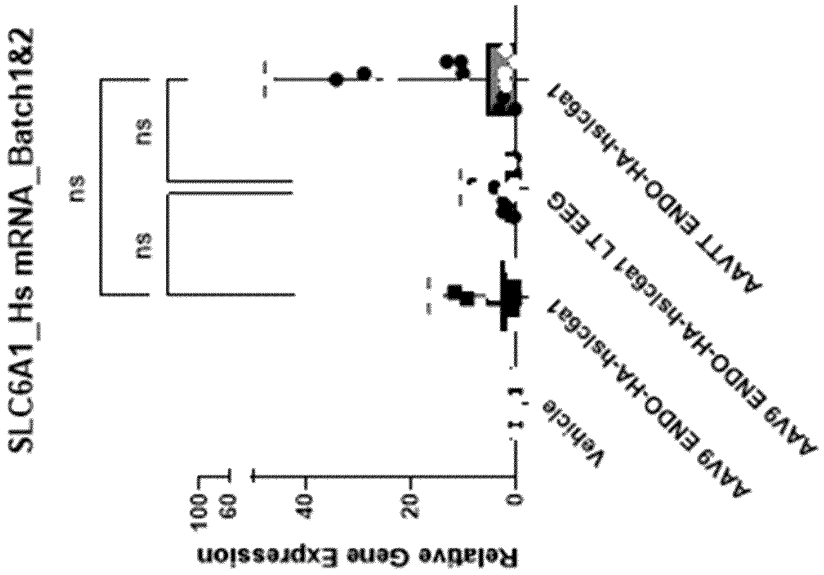

SLC6A1_Hs mRNA_Batch1&2
ns
ns
ns
Relative Gene Expression
100
60
40
20
0
Vehicle
AAV9 ENDO-HA-hslc6a1
AAV9 ENDO-HA-hslc6a1 LT EEG
AAVTT ENDO-HA-hslc6a1

Figure 6

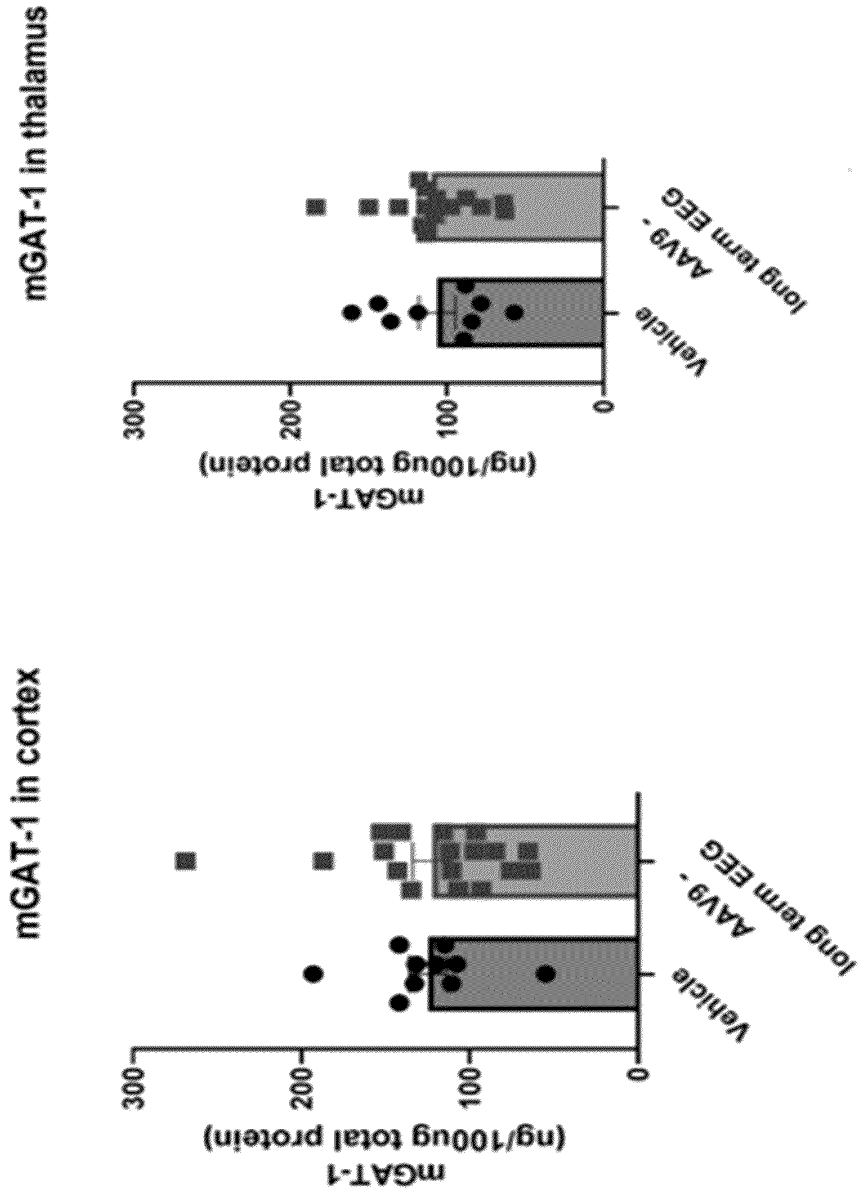
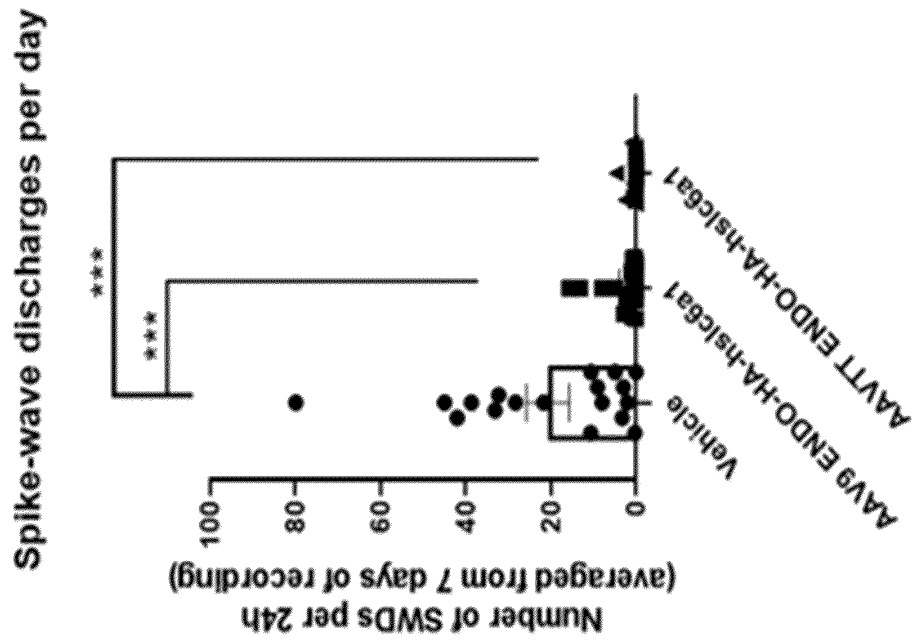

mGAT-1 in thalamus
mGAT-1
(ng/100ug total protein)
300
200
100
0
Vehicle
AAV9 - long term EEG
mGAT-1 in cortex
mGAT-1
(ng/100ug total protein)
300
200
100
0
Vehicle
AAV9 - long term EEG

Figure 7

A


Spike-wave discharges per day
***
***
Number of SWDs per 24h
(averaged from 7 days of recording)
100
80
60
40
20
0
Vehicle
AAV9 ENDO-HA-hslc6a1
AAVTT ENDO-HA-hslc6a1
AAVTT ENDO-HA-hslc6a1

B

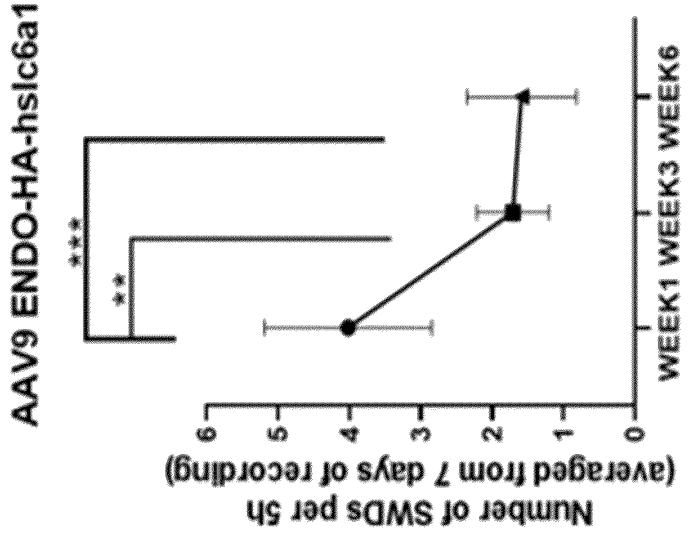

AAV9 ENDO-HA-hslc6a1
***
**
Number of SWDs per 5h
(averaged from 7 days of recording)
6
5
4
3
2
1
0
WEEK1 WEEK3 WEEK6

Figure 8

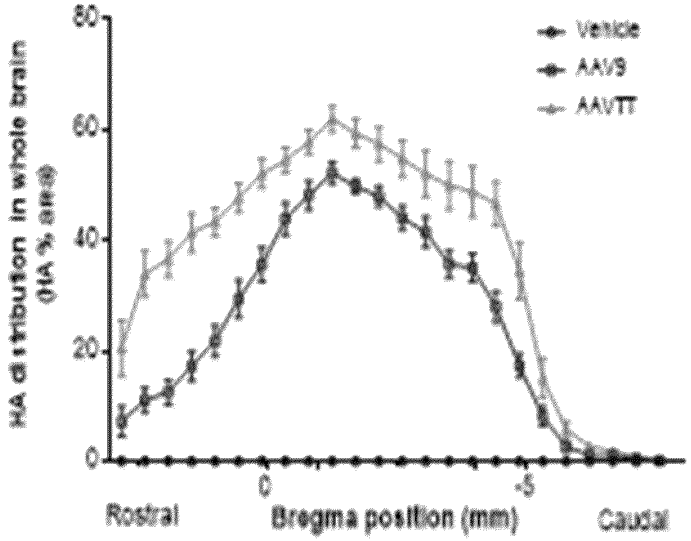

Vehicle
AAV9
AAVTT
HA distribution in whole brain
(HA % area)
80
60
40
20
0
0
-5
Rostral
Bregma position (mm)
Caudal

Figure 8 continued

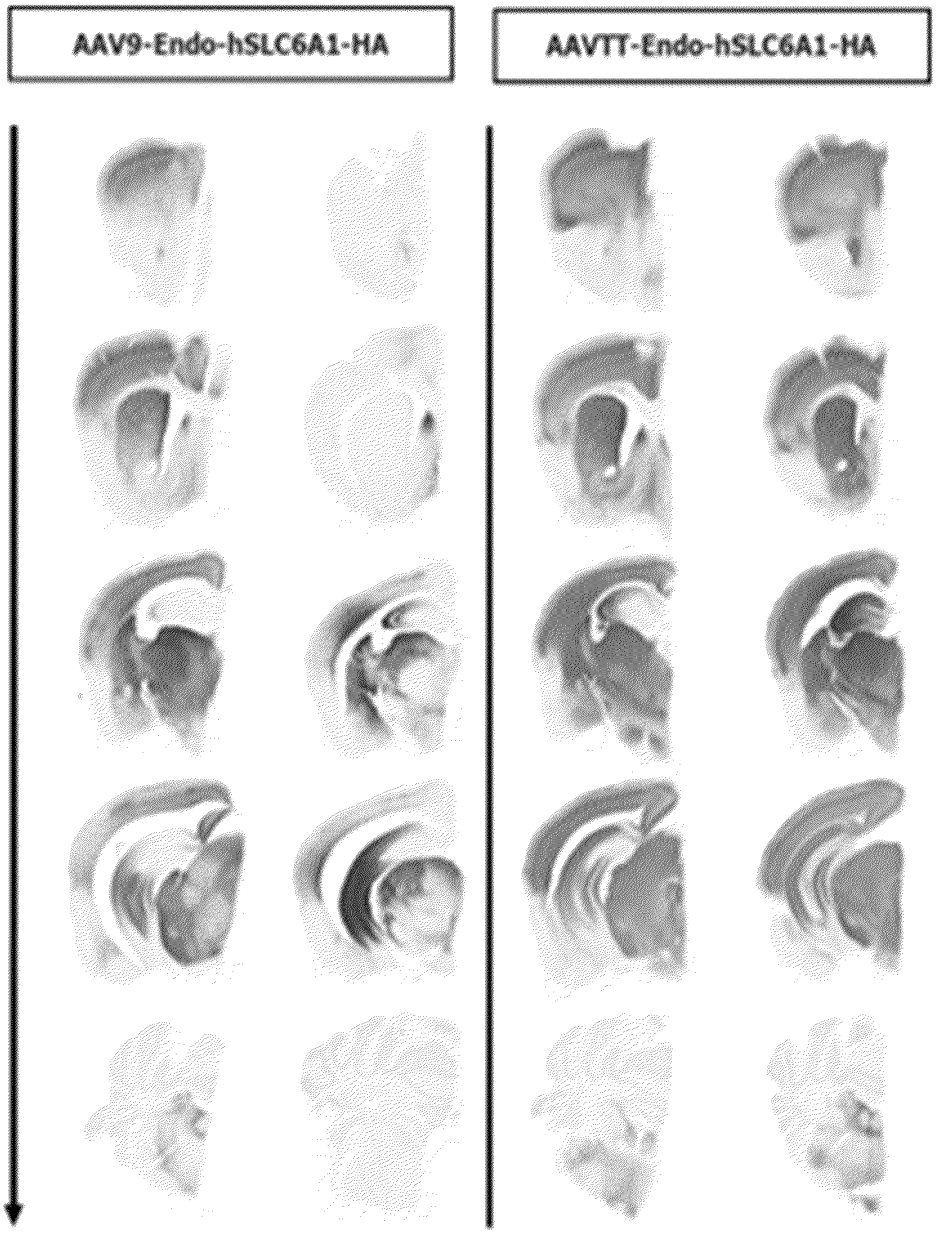


Figure 9

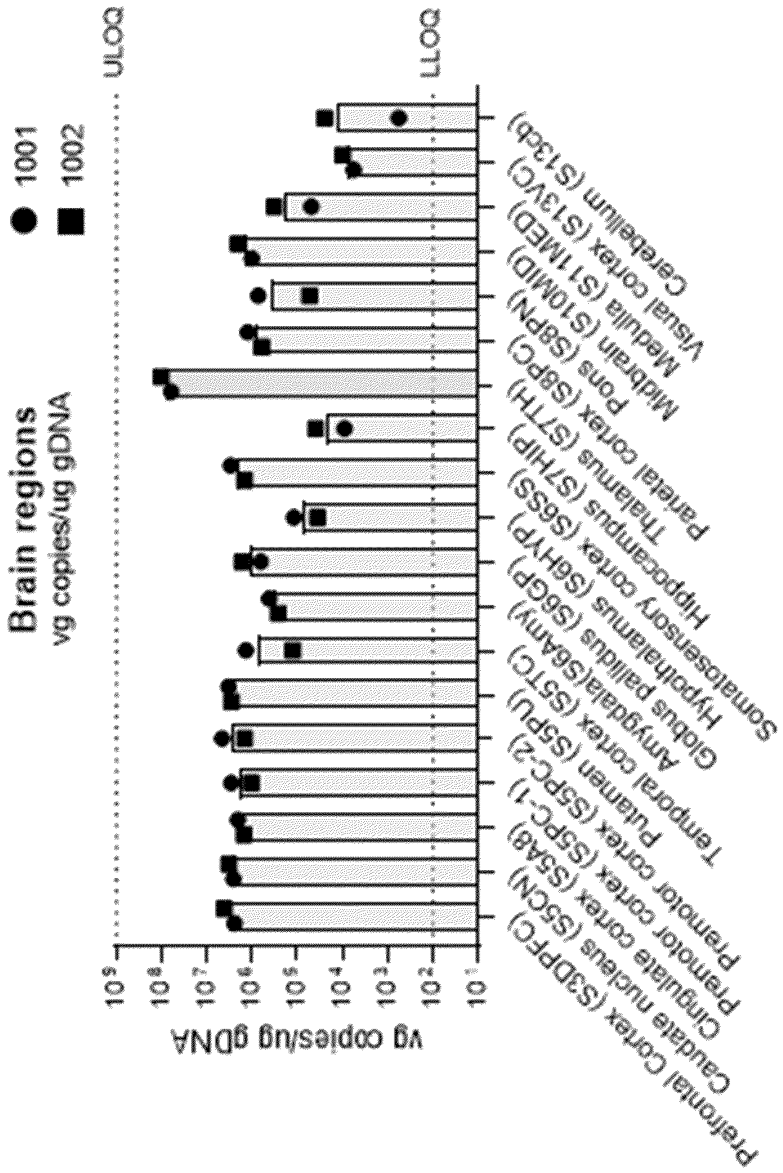
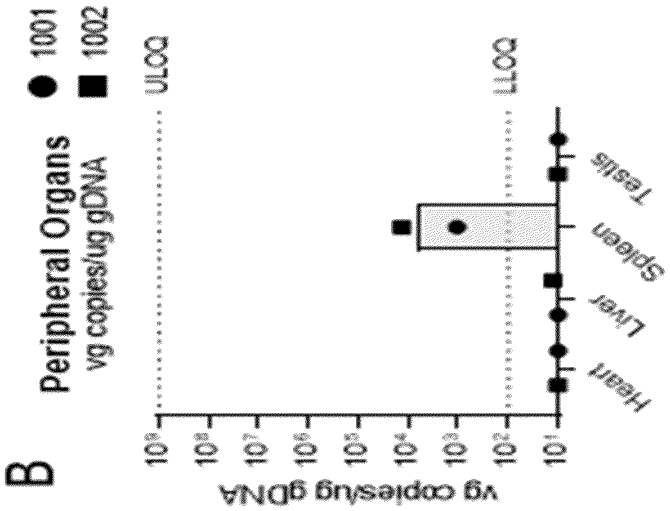

ULOQ
LLOQ
1001
1002
Brain regions
vg copies/ug gDNA
vg copies/ug gDNA
Prefrontal Cortex (S3DPFC)
Caudate nucleus (S5CN)
Cingulate cortex (S5A8)
Premotor cortex (S5PC-1)
Putamen (S5PU)
Temporal cortex (S5TC)
Amygdala(S6AMY)
Globus pallidus (S6GP)
Hypothalamus (S6HYP)
Somatosensory cortex (S6SS)
Hippocampus (S7HIP)
Thalamus (S7TH)
Parietal cortex (S8PC)
Pons (S8PN)
Midbrain (S10MID)
Medulla (S11MED)
Visual cortex (S13VC)
Cerebellum (S13cb)

Figure 10


B
Peripheral Organs
vg copies/ug gDNA
1001
1002
ULOQ
LLOQ
vg copies/ug gDNA
Heart
Liver
Spleen
Testis

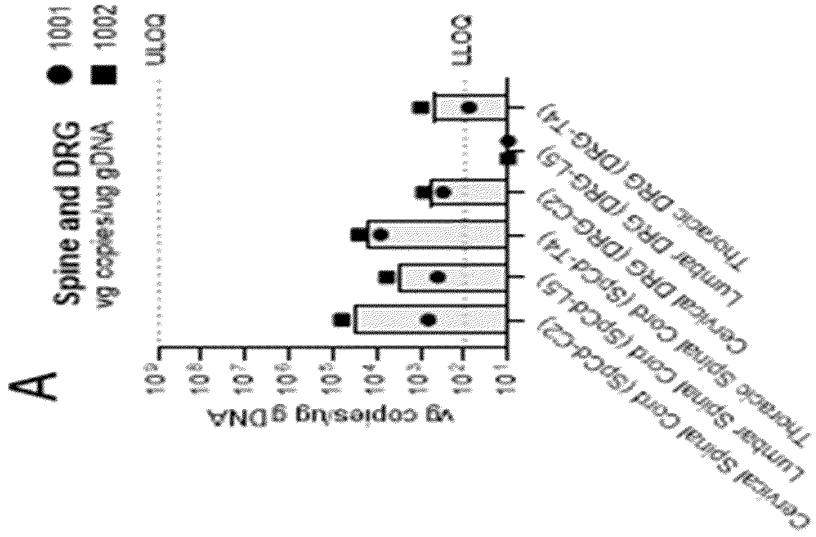

A
Spine and DRG
vg copies/ug gDNA
1001
1002
ULOQ
LLOQ
vg copies/ug gDNA
Cervical Spinal Cord (SpCd-C2)
Lumbar Spinal Cord (SpCd-L5)
Thoracic Spinal Cord (SpCd-T4)
Cervical DRG (DRG-C2)
Lumbar DRG (DRG-L5)
Thoracic DRG (DRG-T4)

Figure 10 continued

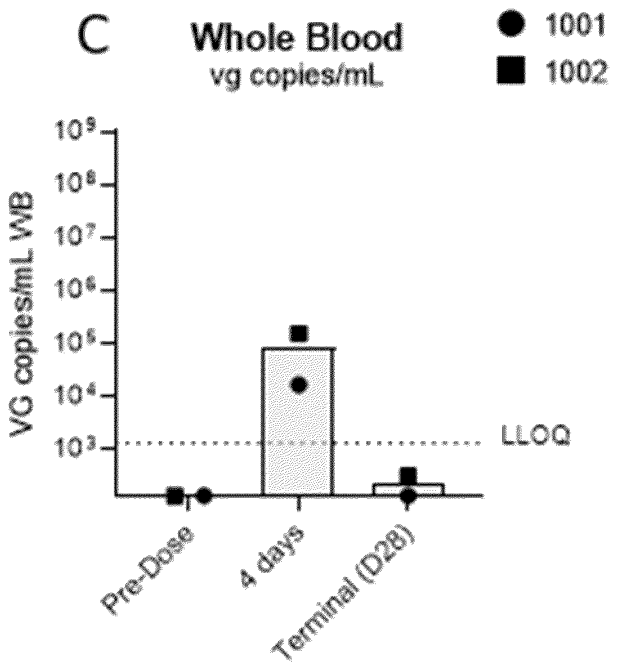


Figure 11

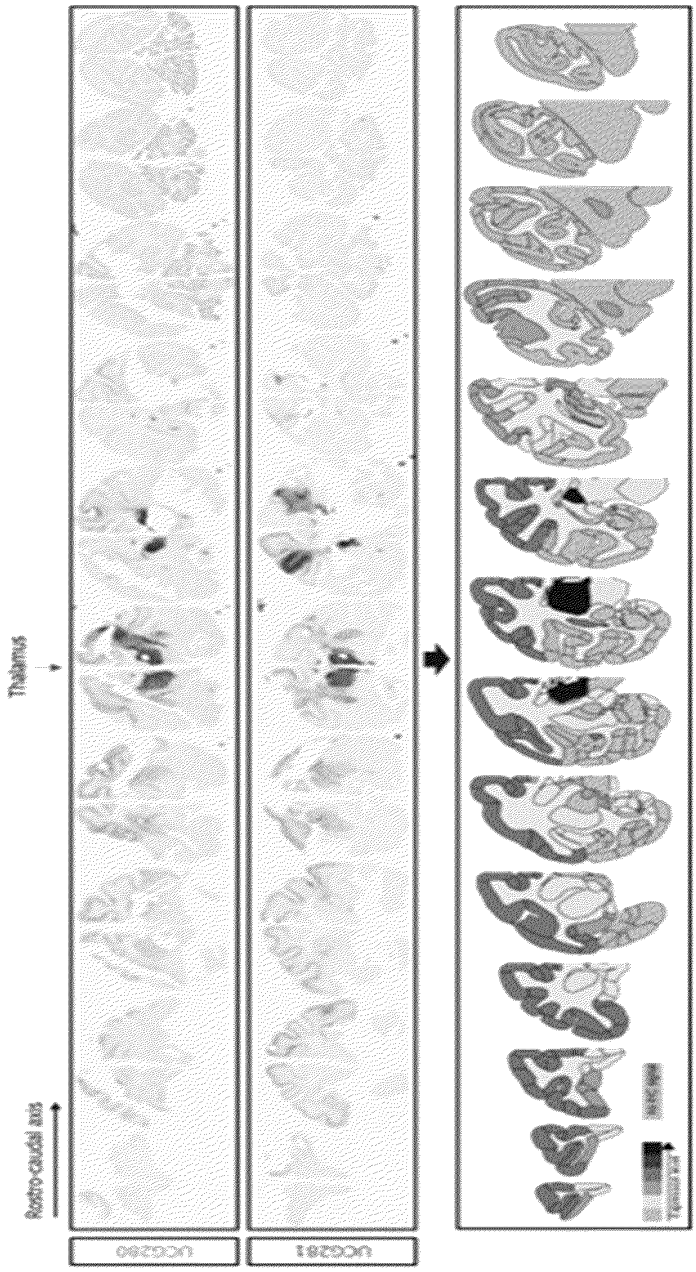


Figure 12

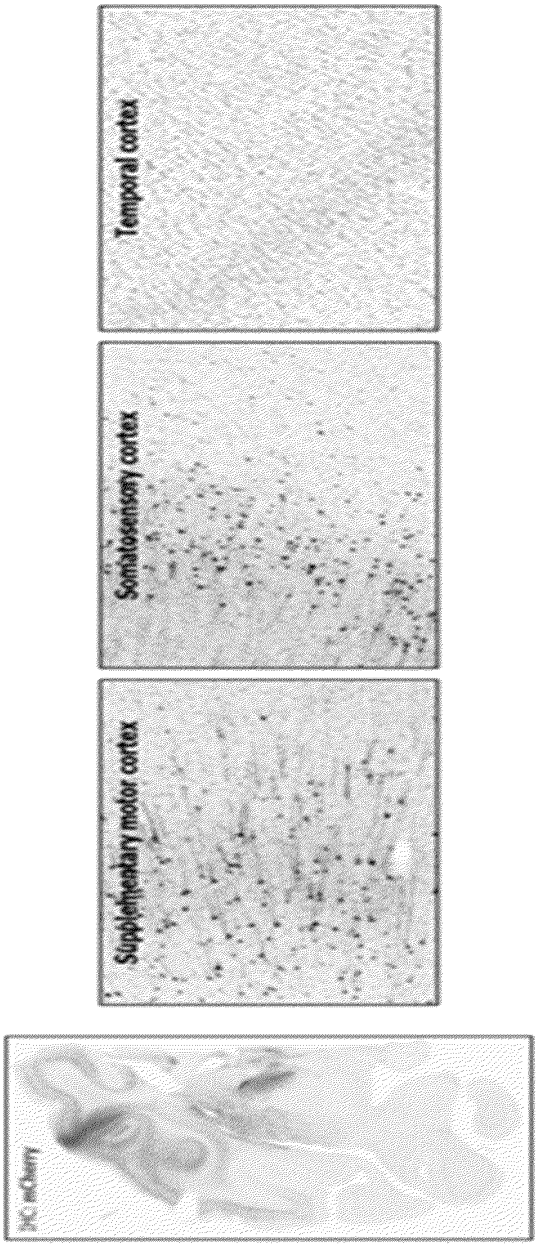

Temporal cortex
Somatosensory cortex
Supplementary motor cortex
hVC: mCherry

Figure 12 continued

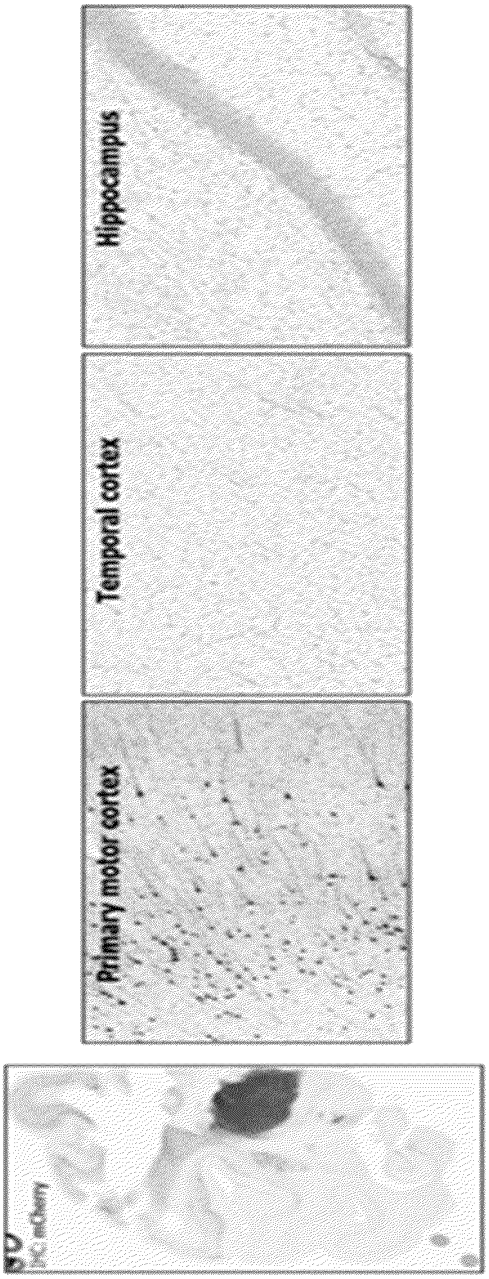
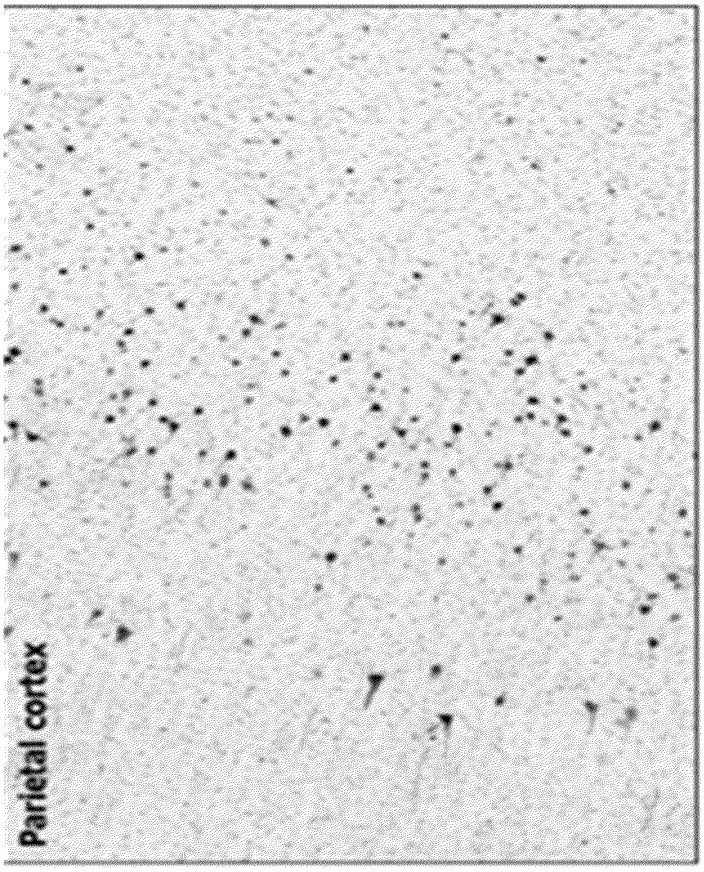


Figure 12 continued



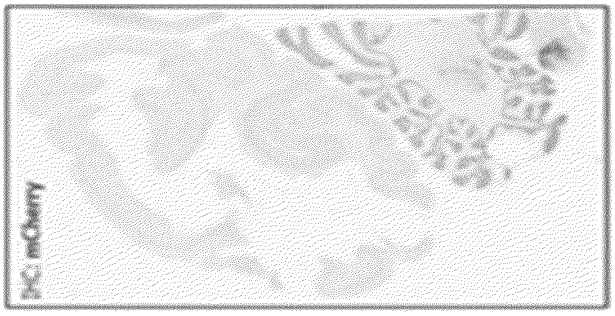


Figure 13

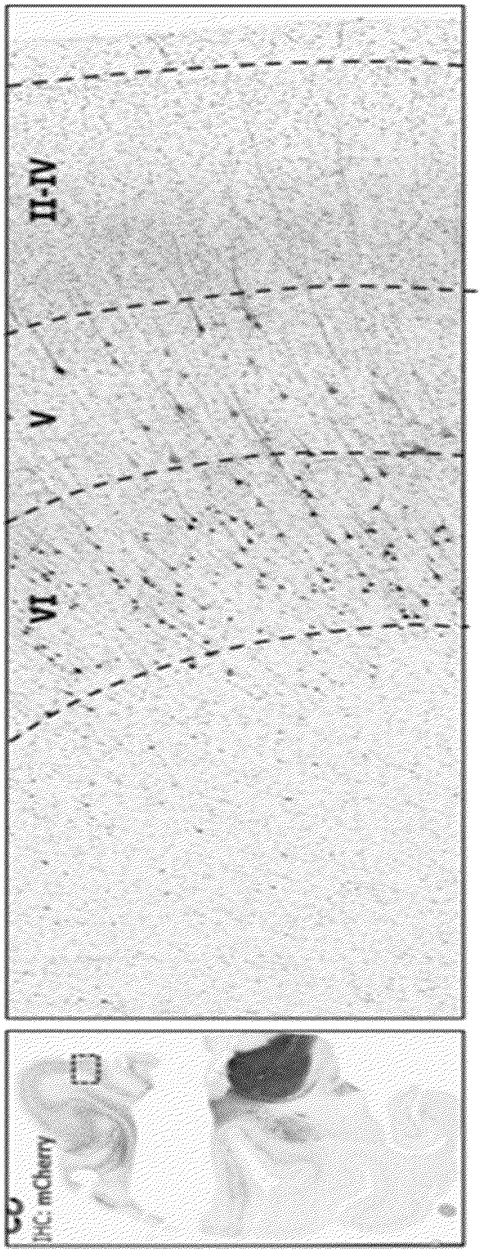


Figure 14

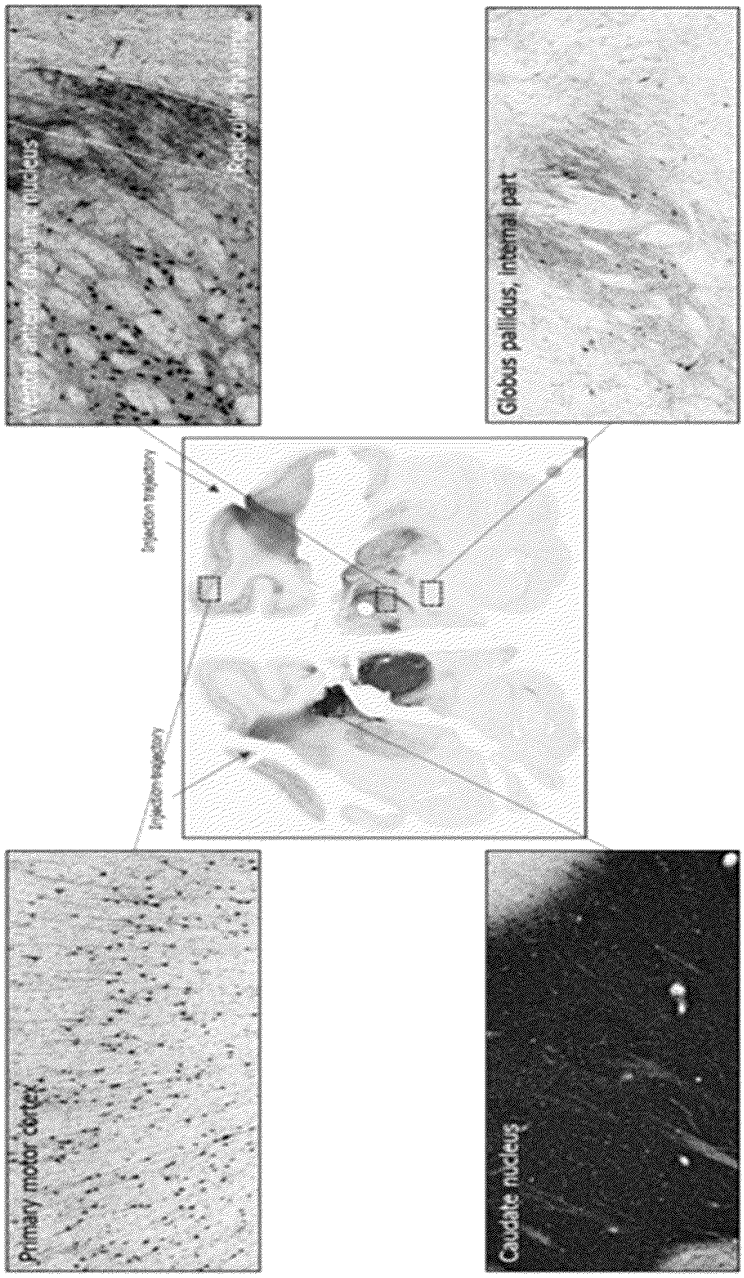

Ventral anterior thalamic nucleus
Reticular thalamic
Globus pallidus, internal part
Injection trajectory
Injection trajectory
Primary motor cortex
Caudate nucleus

Figure 15

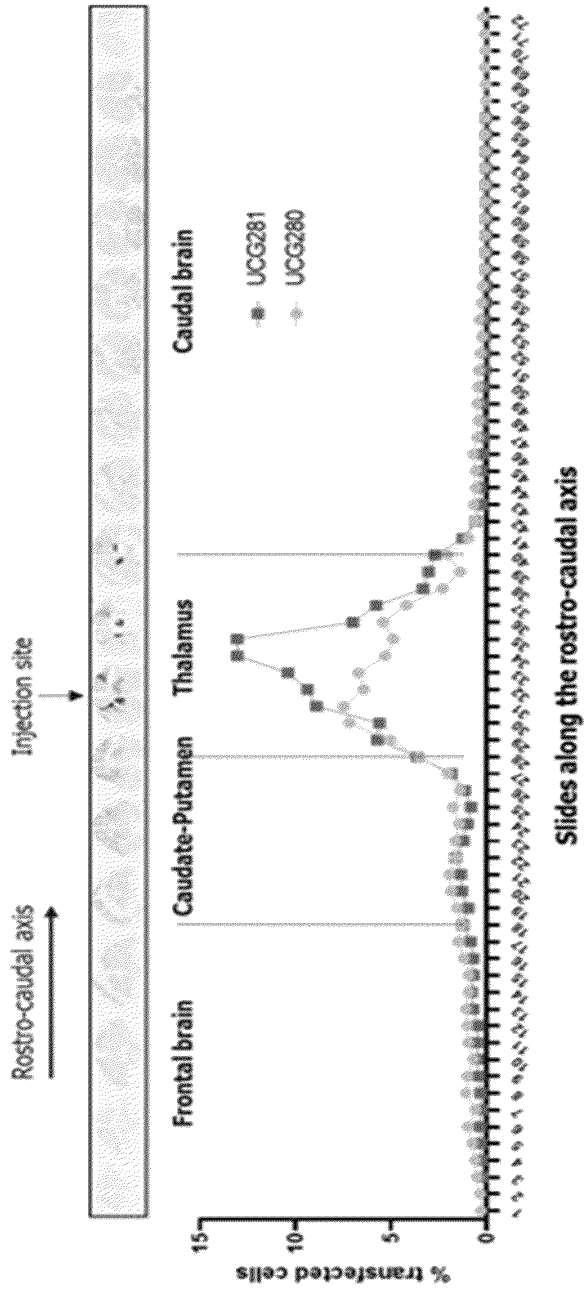
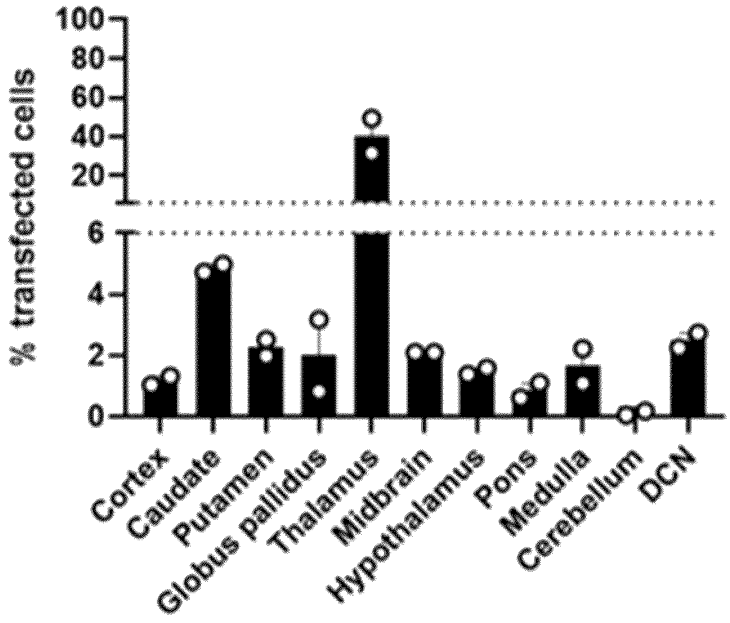

Rostro-caudal axis
Injection site
Frontal brain
Caudate-Putamen
Thalamus
Caudal brain
UCG281
UCG280
% transfected cells
15
10
5
0
Slides along the rostro-caudal axis

Figure 16


% transfected cells
100
80
60
40
20
6
4
2
0
Cortex
Caudate
Putamen
Globus pallidus
Thalamus
Midbrain
Hypothalamus
Pons
Medulla
Cerebellum
DCN

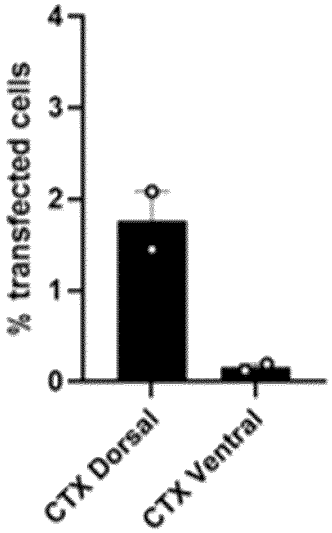

% transfected cells
4
3
2
1
0
CTX Dorsal
CTX Ventral

Figure 17

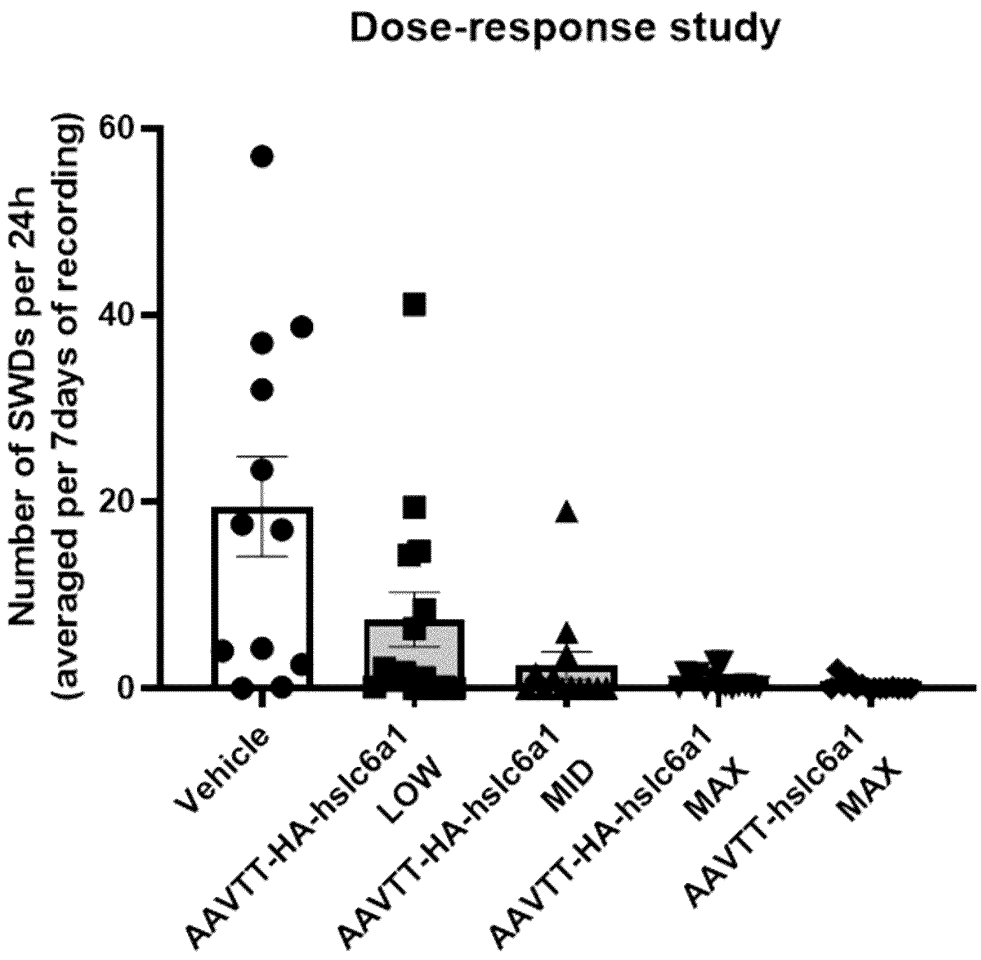

Dose-response study
Number of SWDs per 24h
(averaged per 7days of recording)
60
40
20
0
Vehicle
AAVTT-HA-hslc6a1
LOW
AAVTT-HA-hslc6a1
MID
AAVTT-HA-hslc6a1
MAX
AAVTT-hslc6a1
MAX

Figure 18

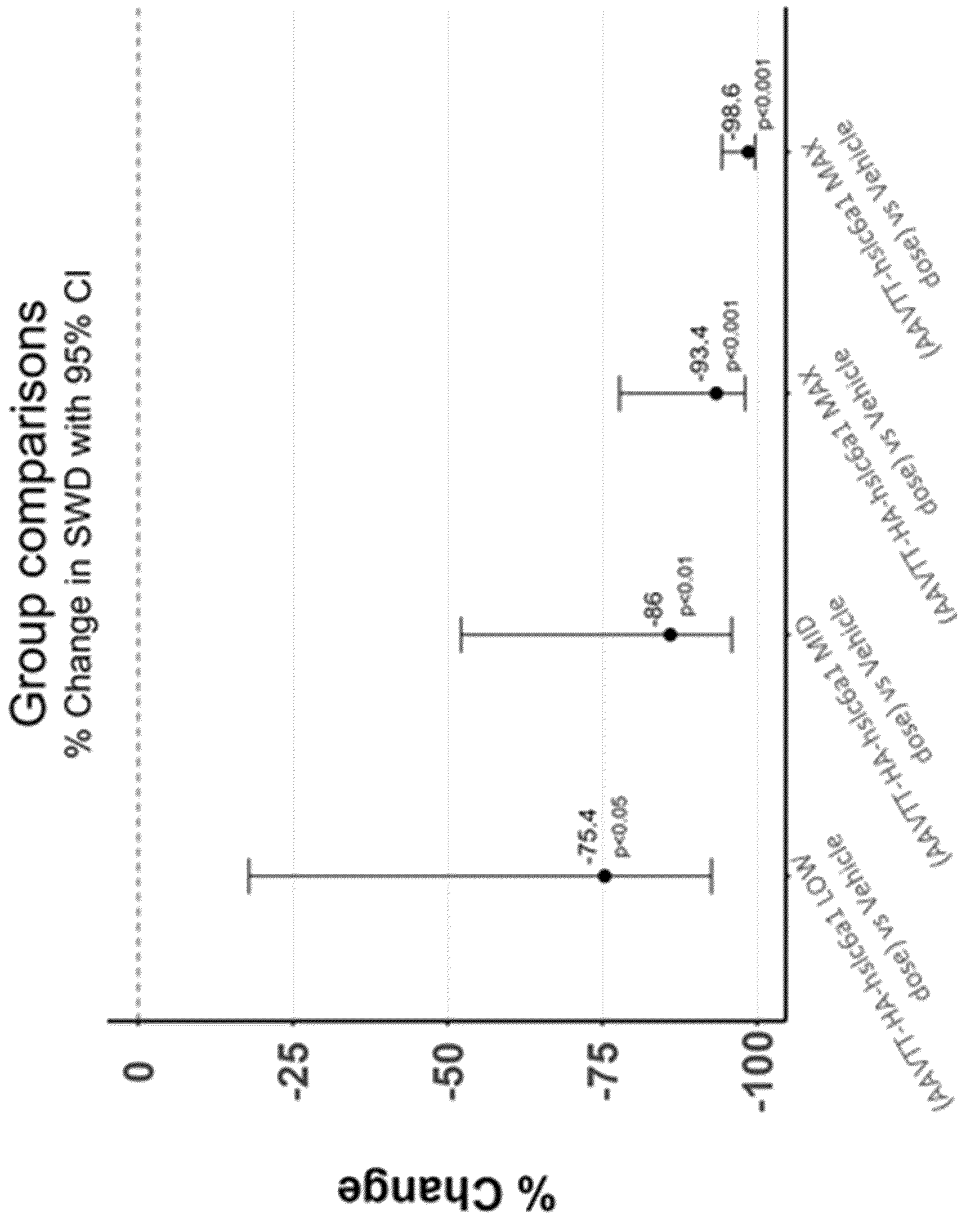

Group comparisons
% Change in SWD with 95% CI
% Change
0
-25
-50
-75
-100
-75.4
p<0.05
-86
p<0.01
-93.4
p<0.001
-98.6
p<0.001
(AAVTT-HA-hsic6a1 LOW dose) vs Vehicle
(AAVTT-HA-hsic6a1 MID dose) vs Vehicle
(AAVTT-HA-hsic6a1 MAX dose) vs Vehicle
(AAVTT-hsic6a1 MAX dose) vs Vehicle

Figure 19

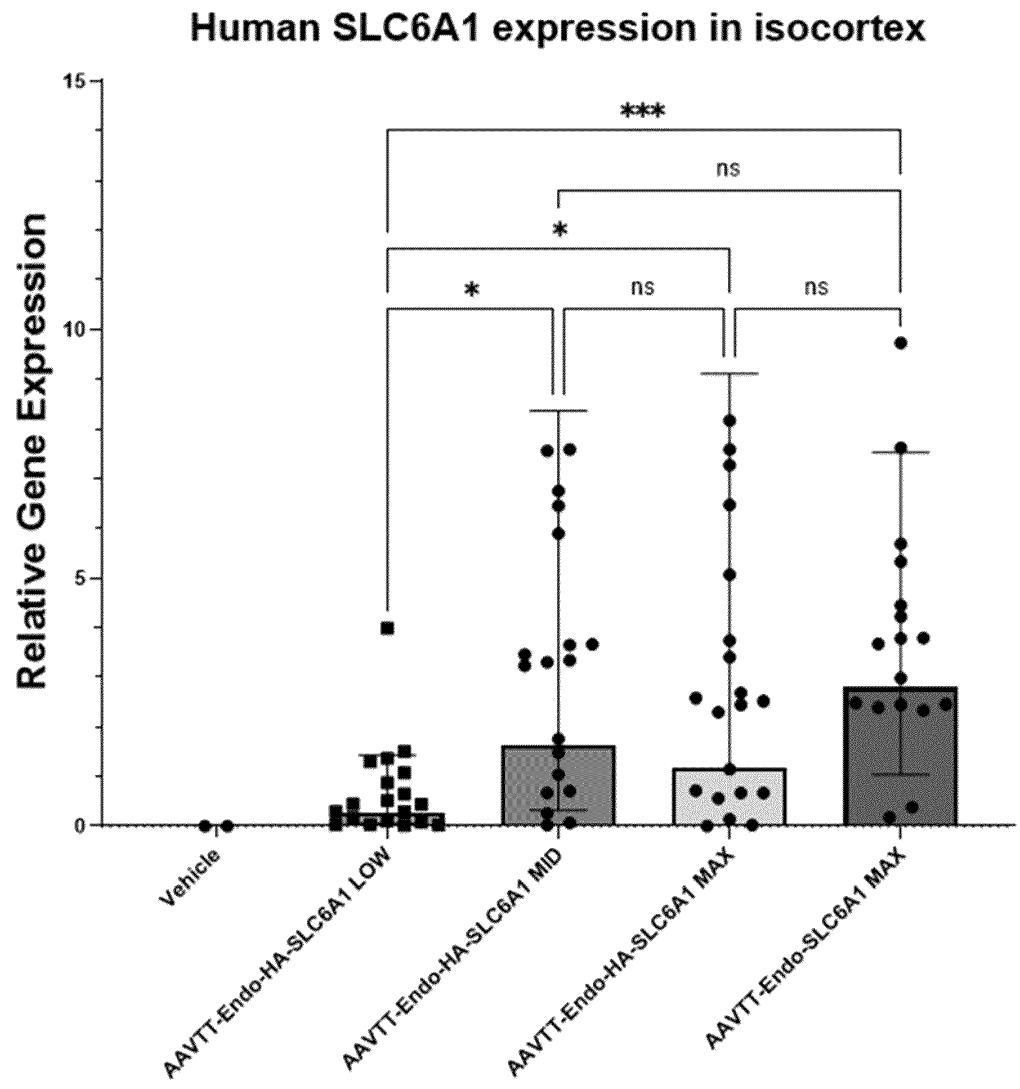

Human SLC6A1 expression in isocortex
Relative Gene Expression
15
10
5
0
***
ns
*
*
ns
ns
Vehicle
AAVTT-Endo-HA-SLC6A1 LOW
AAVTT-Endo-HA-SLC6A1 MID
AAVTT-Endo-HA-SLC6A1 MAX
AAVTT-Endo-SLC6A1 MAX

Figure 19 continued

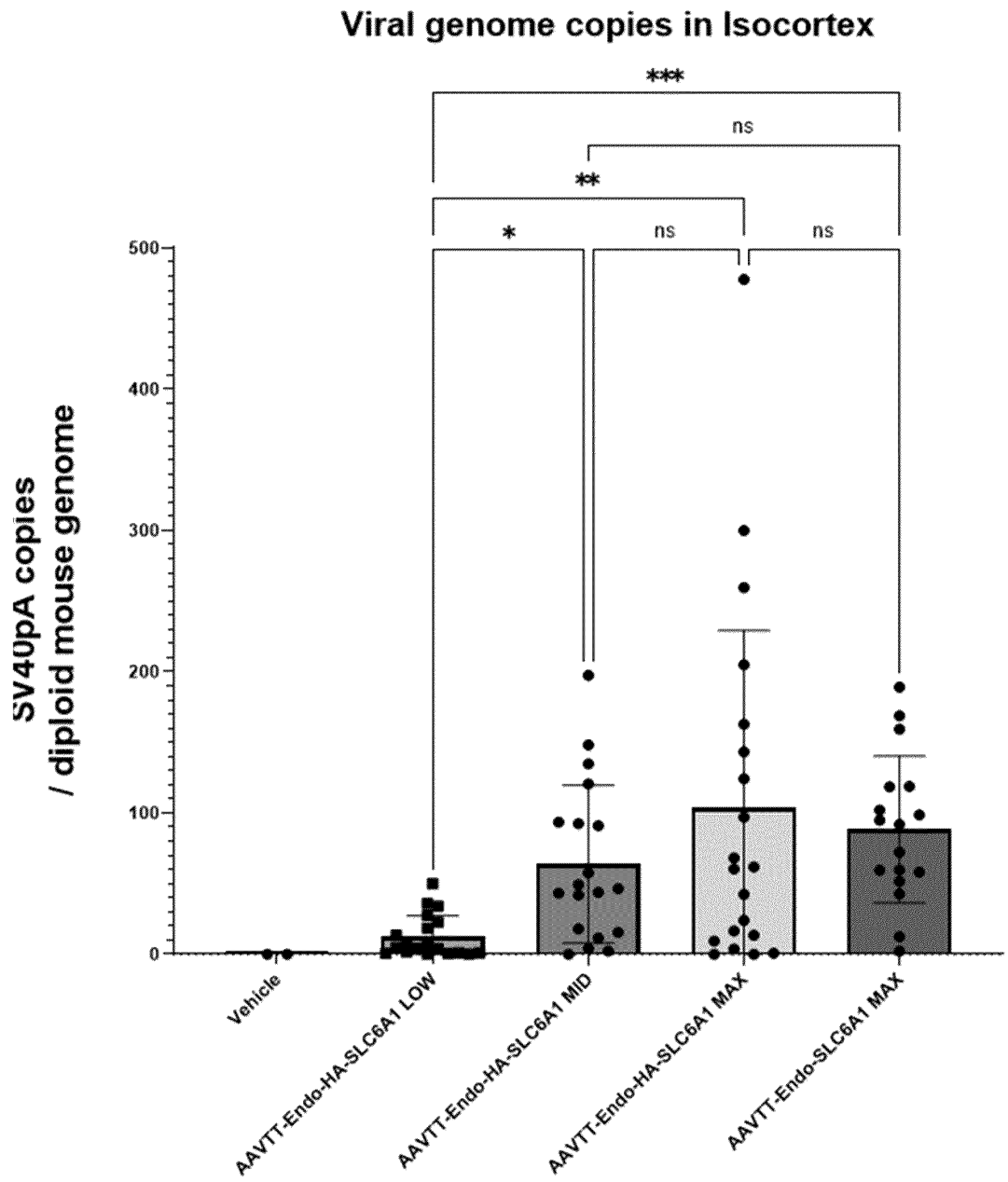


Figure 20

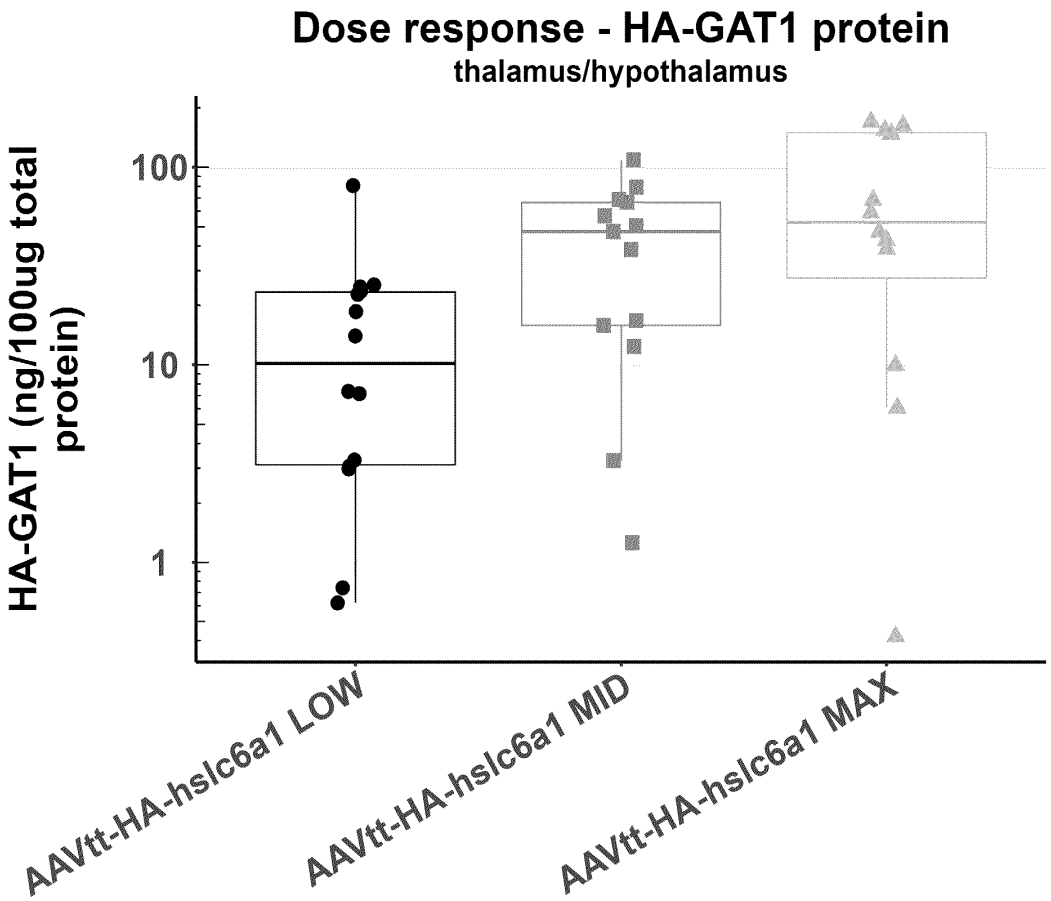

Dose response - HA-GAT1 protein
thalamus/hypothalamus
HA-GAT1 (ng/100ug total protein)
100
10
1
AAVtt-HA-hslc6a1 LOW
AAVtt-HA-hslc6a1 MID
AAVtt-HA-hslc6a1 MAX

Figure 20 continued

