# Supplementary material

| Gene | Primers | Product | GenBank ID |
|---|---|---|---|
| **Rat ChAT** | Forward: 5′-TTA ATT TCC GCC GTC TC-3′ | 446 bp | **290567** |
| | Reverse: 5′-GCT TCT TGT TGC TTG TC-3′ | | |
| **Mouse $5HT_{1A}$** | Forward: 5′-GGA CAG GCG GCA ACG ATA C-3′ | 570 bp | **15550** |
| | Reverse: 5′-GCA GCA GCG GAA TAT AGA AAG C-3′ | | |
| **Mouse $5HT_{2B}$** | Forward: 5'- GAT TGT TTG TGA TGC CGA TTG C-3' | 419 bp | **15559** |
| | Reverse: 5'- AGT GTG AAT GGT GAG AAA GTA AGT G -3' | | |
| ***Xenopus* $5HT_{1A}$** | Forward: 5'- TAC CTC TTG GAA CAT ACT TC -3' | 106 bp | **378605** |
| | Reverse: 5'- AGA GAA TGA GCG TCC TAG -3' | | |
| ***Xenopus* $5HT_{2B}$** | Forward: 5'- AAC ACC ACC TTC AGC GCA AGC -3' | 336 bp | **398699** |
| | Reverse: 5'- ACC AAC ACT GGA TAG TCT GCG-3' | | |
| **Mouse GAPDH** | Forward: 5'- ACC ACA GTC CAT GCC ATC AC -3' | 452 bp | **14433** |
| | Reverse: 5'- TCC ACC ACC CTG TTG CTG TA -3' | | |

**$5HT_{1A}$** ***Xenopus*** amplification was performed using the following profile:
35 PCR cycle for 95 °C for 40 sec, 51 °C for 40 sec, 72 °C for 30 sec.
**$5HT_{1A}$ mouse** amplification was performed using the following profile:
32 PCR cycle for 95 °C for 40 sec, 61 °C for 40 sec, 72 °C for 40 sec
**$5HT_{2B}$** ***Xenopus*** amplification was performed using the following profile:
30 PCR cycle for 95 °C for 40 sec, 60 °C for 40 sec, 72 °C for 40 sec.
**$5HT_{2B}$ mouse** amplification was performed using the following profile:
32 PCR cycle for 95 °C for 40 sec, 60 °C for 40 sec, 72 °C for 40 sec.
**ChAT** amplification was performed using the following profile:
30 PCR cycle for 95 °C for 40 sec, 52°C for 40 sec, 72 °C for 40 sec.
**GAPDH** (glyceraldehyde-3-phosphate dehydrogenase) amplification was performed using the following profile:
18 PCR cycles for 95 °C for 40 sec, 66 °C for 40 sec, 72 °C for 40 sec.

**Table S1**: Primers and thermal cycling conditions used in RT-PCR.

| Gene | Primers | Product | GenBank ID |
|---|---|---|---|
| **EGR-1** | Forward: 5'- TCC GAC CTC TTC ATC CTC-3' | 149 bp | **13653** |
| | Reverse: 5'- ATG TCA GTG TTG GGA GTA G-3' | | |
| **β-actin** | Forward: 5'-CCC AGG CAT TGC TGA CAG G-3' | 143 bp | **11461** |
| | Reverse: 5'-GCT GGA AGG TGG ACA GTG AG-3' | | |

Thermal cycling conditions consisted of an initial denaturation step at 95°C for 3 min followed by 45 cycles at 95°C for 30 sec. Annealing temperature was 55°C for both EGR-1 and β-actin. Two cycles were included as final steps: one at 95°C and the other at the corresponding annealing temperature of each tested gene, both for 1 min.

**Table S2**: Primers and thermal cycling conditions used in Real Time-PCR assay.

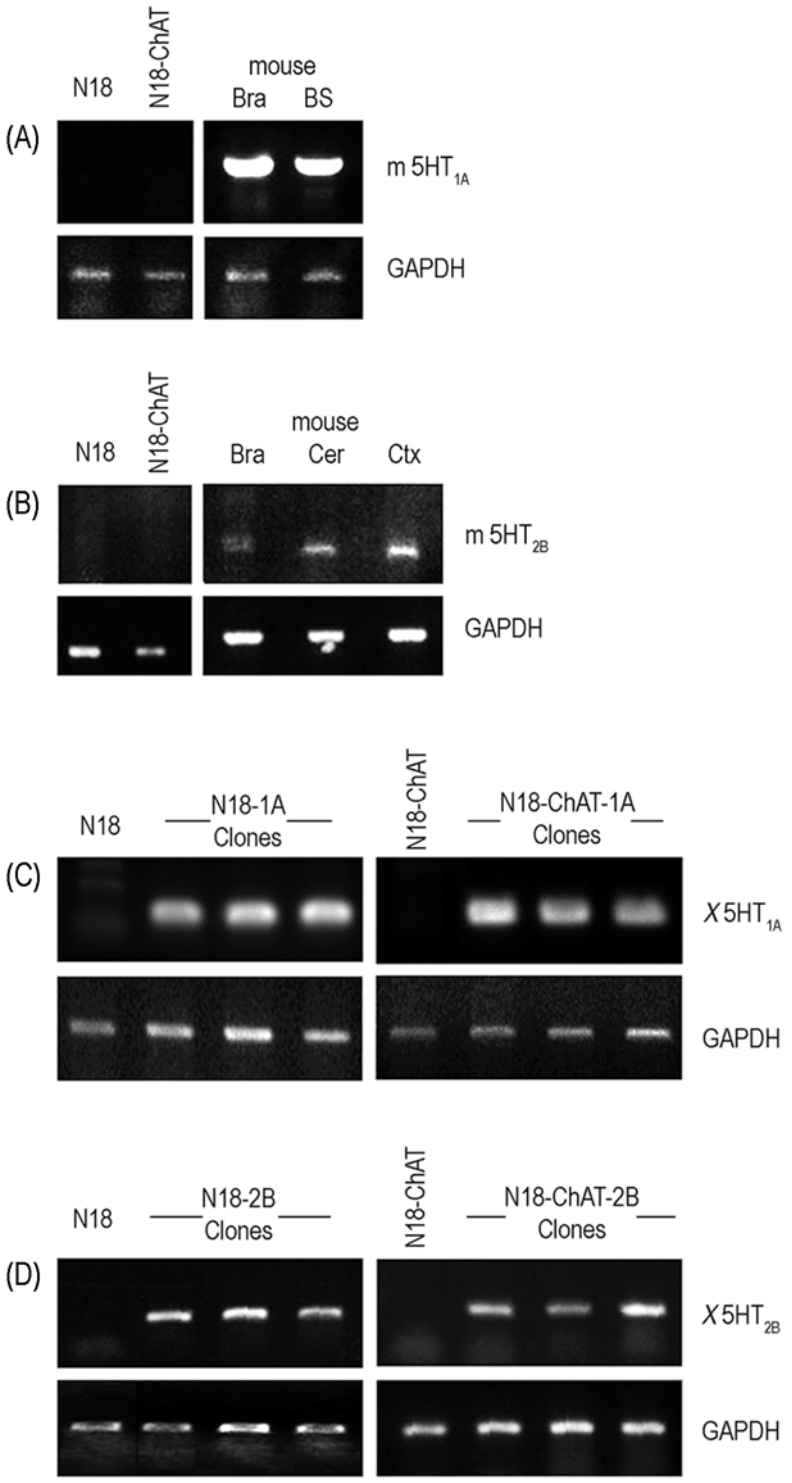
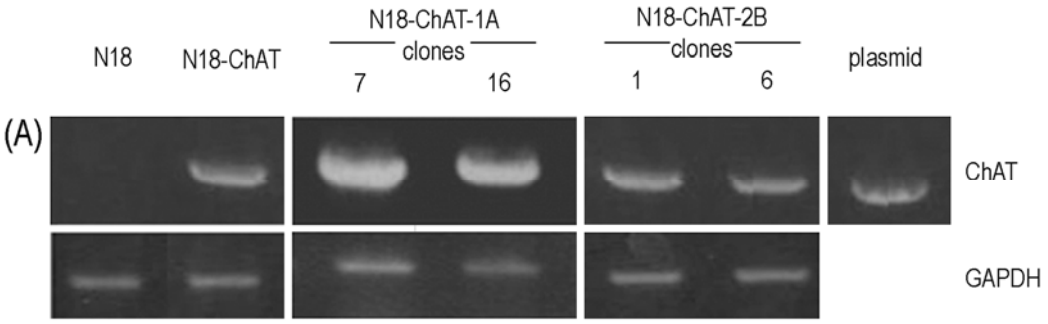



**Fig. S1**: **Panel A:** RT-PCR using mouse $5HT_{1A}$ and mouse GAPDH primers in N18 and N18-ChAT cells, mouse brain (Bra) and mouse brain stem (B.S.). **Panel B:** RT-PCR using mouse $5HT_{2B}$ and mouse GAPDH primers in N18 and N18-ChAT cells, mouse brain (Bra), cerebellum (Cer) and Cortex (Ctx). **Panel C:** RT-PCR using *Xenopus* $5HT_{1A}$ and mouse GAPDH primers in N18, N18-1A, N18-ChAT and N18-ChAT-1A cells. **Panel D:** RT-PCR using *Xenopus* $5HT_{2B}$ and mouse GAPDH primers in N18, N18-2B, N18-ChAT and N18-ChAT-2B cells.




(B)

| ChAT Activity (nmol ACh syntesized/mg prot) | |
|---|---|
| mouse brain | 1,10±0,18 |
| N18 | N.D. |
| N18-ChAT | 18,86±1,78 |
| N18-ChAT-1A 7 clone | 2,27±0,47 |
| N18-ChAT-1A 16 clone | 2,04±0,24 |
| N18-ChAT-2B 1clone | 8,15±0,06 |
| N18-ChAT-2B 6 clone | 14,12±2,47 |

**Fig. S2**: **Panel A:** RT-PCR using rat ChAT and mouse GAPDH primers in N18, N18-ChAT, N18-ChAT-1A and N18-ChAT-2B cells and plasmid (construct containing ChAT gene). **Panel B:** ChAT specific activity was measured in extracts of mouse brain, N18, N18-ChAT, N18-ChAT-1A and N18-ChAT-2B clones. Data are mean±SEM values of at least six independent experiments done at least on triplicate cultures. N.D., not detectable.