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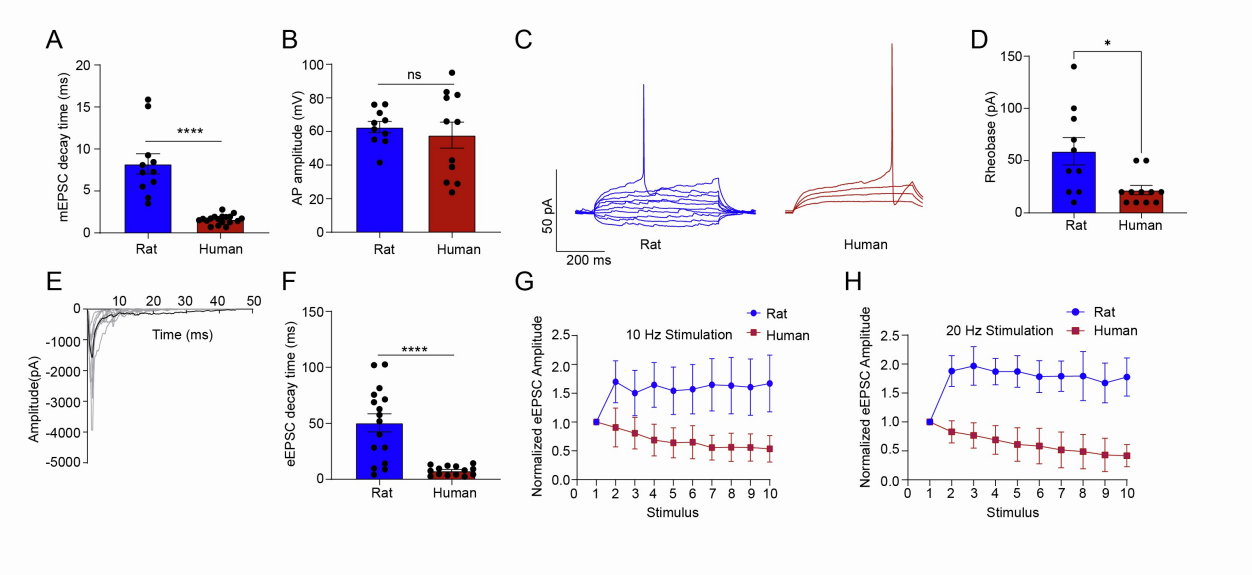
Supplemental information

Neurotransmitter release progressively desynchronizes in induced human neurons during synapse maturation and aging

Burak Uzay, Aiden Houcek, Z. Zack Ma, Christine Konradi, Lisa M. Monteggia, and Ege T. Kavalali



Supplemental Figure S1. Comparison of Electrophysiological Properties of Human iN Cells and Rat Hippocampal Neurons, Related to Figure 1

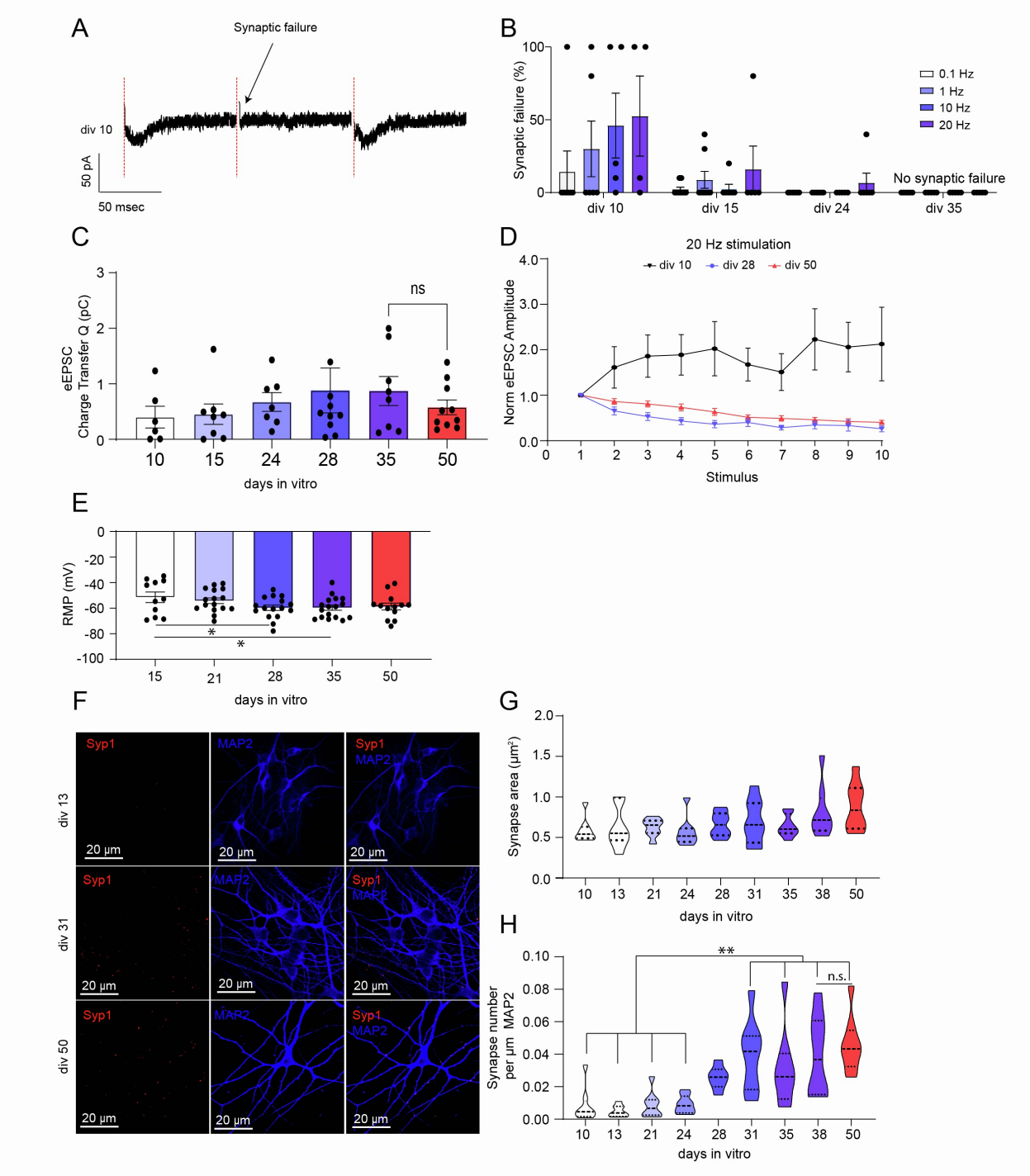
(A) Human iN cells demonstrate sharper miniature event kinetics where mEPSC decay time is significantly smaller compared to rat hippocampal neurons ; dots show individual cells; bars show mean±SEM; N=3; human iN cells, n=18; rat hippocampal cells, n=11; $p<0.0001$; Unpaired t-test.

(B)There is not a significant difference between AP amplitudes in human iN cells and rat hippocampal neurons ; dots show individual cells; bars show mean±SEM; N=3 ; human iN cells, n=11; rat hippocampal cells, n=10; n=0.5913; Unpaired t-test.

(C-D) Representative traces of stepwise current injection and rheobase measurements show that human iN cells are more excitable compared to rat hippocampal neurons ; dots show individual cells; bars show mean±SEM; N=2; human iN cells, n=11; rat hippocampal cells, n=10; $p=0.0116$; Unpaired t-test.

(E-F) Human iN cells have a significantly smaller eEPSC decay time where the recording reaches baseline in 10 miliseconds following stimulation; dots show individual cells; bars show mean±SEM; N=3; human iN cells, n=14; rat hippocampal cells, n=16; $p<0.0001$; Unpaired t-test.

(G-H) Upon high frequency (10-20 Hz) stimulation rat hippocampal neurons show synaptic facilitation whereas human iN cells show synaptic depression; bars show mean±SEM;10 Hz: human iN cells, n=9; rat hippocampal cells, n=8; 20 Hz: human iN cells, n=7; rat hippocampal cells, n=8; N=3.



Supplemental Figure S2. Changes in Neurotransmitter Release Properties and Synapse Density through iN cell Maturation, Related to Figure 2

(A-B) Representative traces of synaptic failures at div 10 and quantification of synaptic failure percentages from div 10 to div 35 showing that synaptic failures are more frequent at earlier stages of development; bars show mean±SEM.

(C) eEPSC Charge Transfer (Q) does not change from div 35 to div 50; dots show individual cells; bars show mean±SEM; div 10, n=6; div 15, n=8; div 24, n=7; div 28, n=9; div 35, n=8; div 50, n=10; N=2; p=0.233; One way ANOVA-Fisher's LSD.

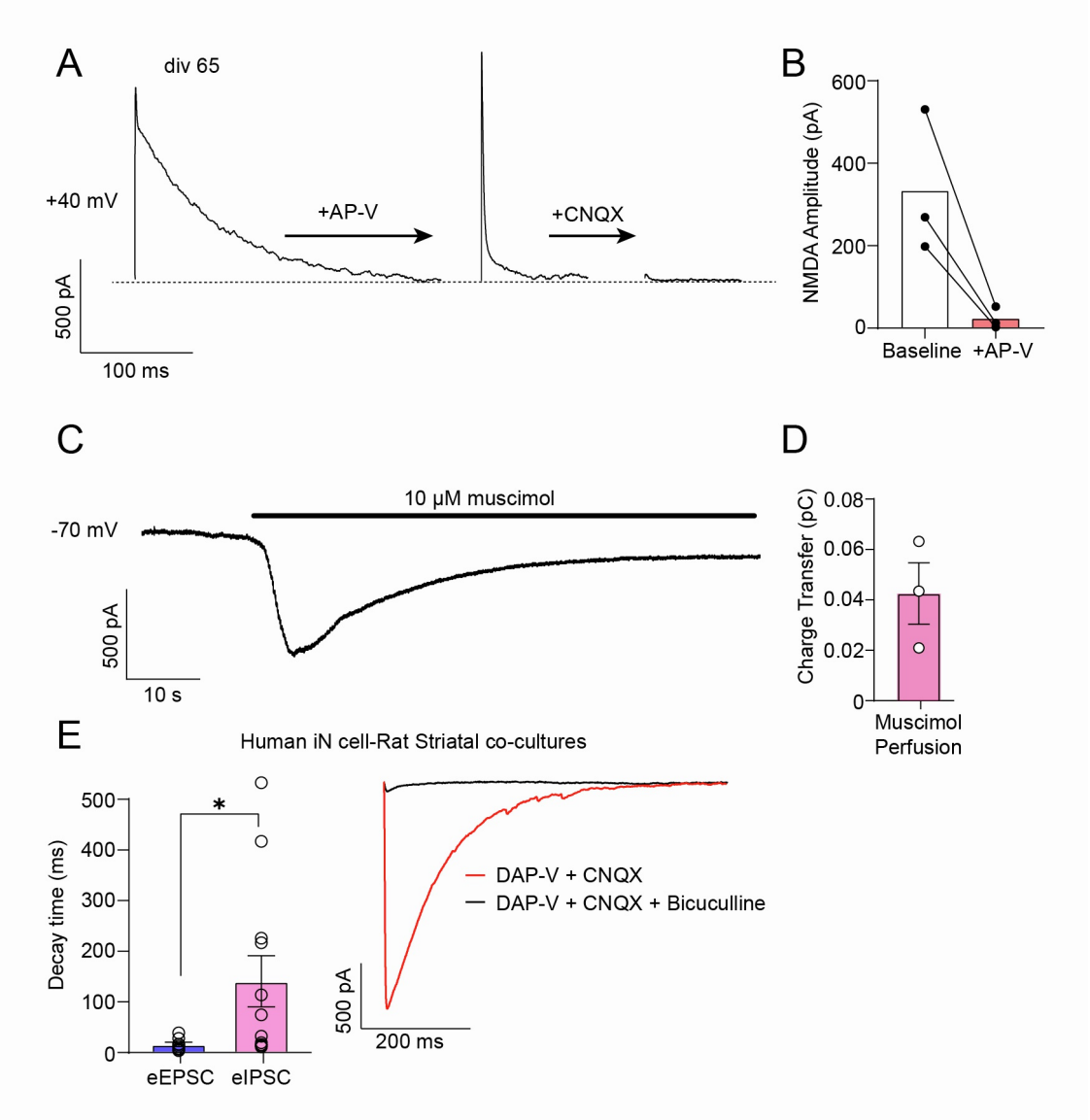
(D)The normalized eEPSC amplitudes upon 20 Hz stimulation at div 10, 28 and 50 showing that eEPSCs facilitate upon stimulation at div 10 but depress at div 28 and at div 50; bars show mean±SEM;div 10 n=; div 28 n=; div 50 n=; N=2.

(E)The resting membrane potential of iN cells become less depolarized from div 15 to div 50; dots show individual cells; bars show mean±SEM; div 15, n=11; div 21, n=16; div 28, n=16; div 35, n=17; div 50, n=13; N=2; p=0.029 , div 15 vs div 28; p=0.027 , div 15 vs div 25; One way ANOVA-Fisher's LSD.

(F)Representative images of immunofluorescent staining of human iN cells for synaptic markers throughout development and maturation (Red:Synapsin1, Blue:MAP2)(Scale: 20µm)

(G-H) The synapse area and synapse density of iN cells at different days in vitro reveal that synapse area does not significantly change whereas synapse density increases from earlier divs (10-14) to later divs (31-50); For each div, n=8; N=1; p<0.001, div 10 vs div 38, please see Supplementary Table S1 for detailed comparisons;One way ANOVA-Fisher's LSD.

Significance levels were stated as follows: *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001. ns denotes non-significance.



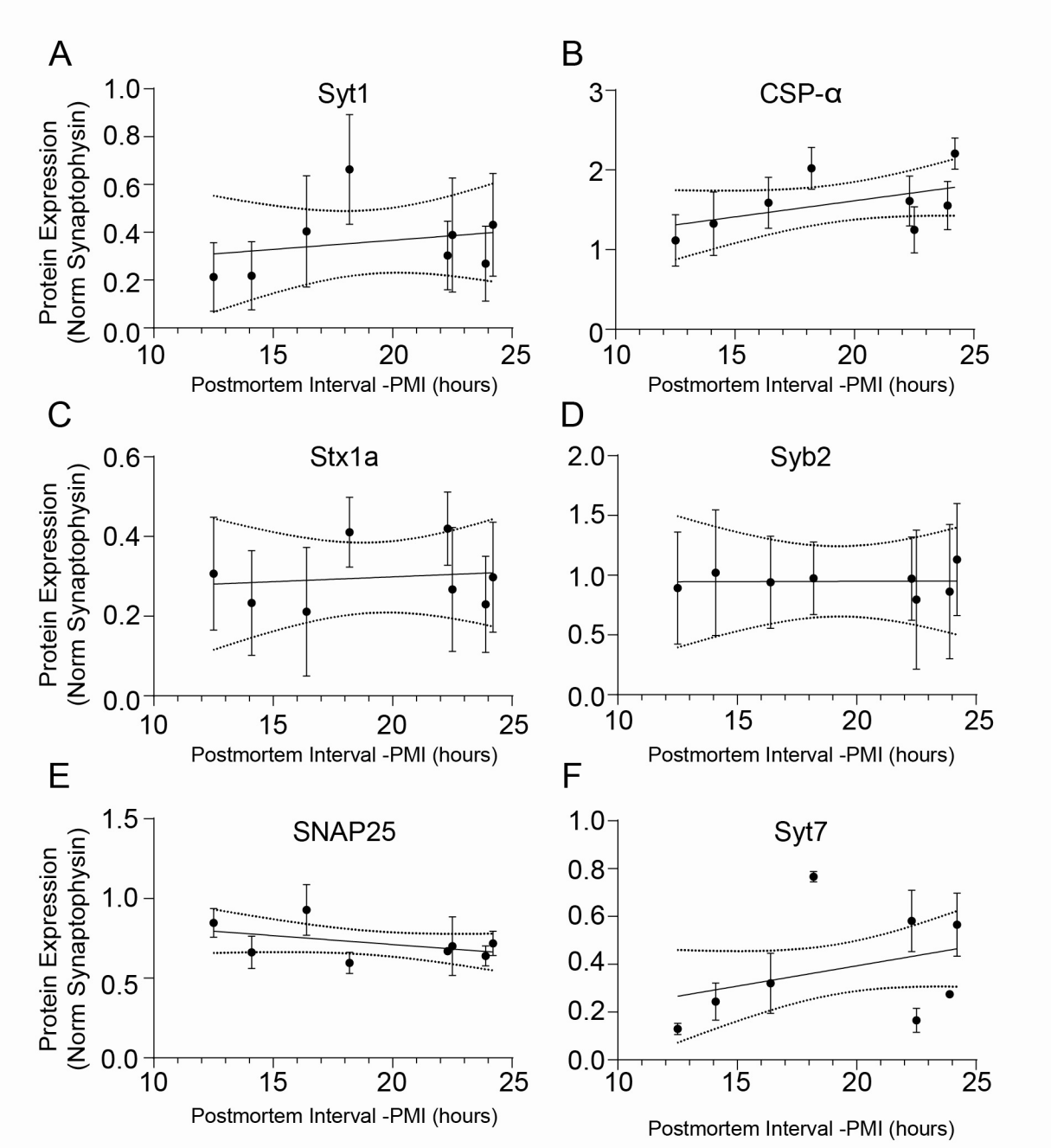
Supplemental Figure S3. Representative Traces of AMPA, NMDA and GABAergic Currents at div 65 iN cells, Related to Figure 3 and Figure 4

(A-B) Representative traces and quantification showing pharmacological blockade of AMPA and NMDA currents at div 65, revealed by the loss of NMDA current upon AP-V perfusion and the loss of AMPA current upon CNQX perfusion; dots show individual cells; n=3; N=1.

(C-D) Representative trace of GABAergic current and quantification of the charge transfer upon 10µM muscimol perfusion confirming the presence of $GABA_A$ receptors on iN cells; dots show individual cells; bars show mean±SEM; n=3; N=1.

(E) When co-cultured with rat striatal neurons, human iN cells demonstrate postsynaptic inhibitory current with slower kinetics compared to eEPSCs dots show individual cells; bars

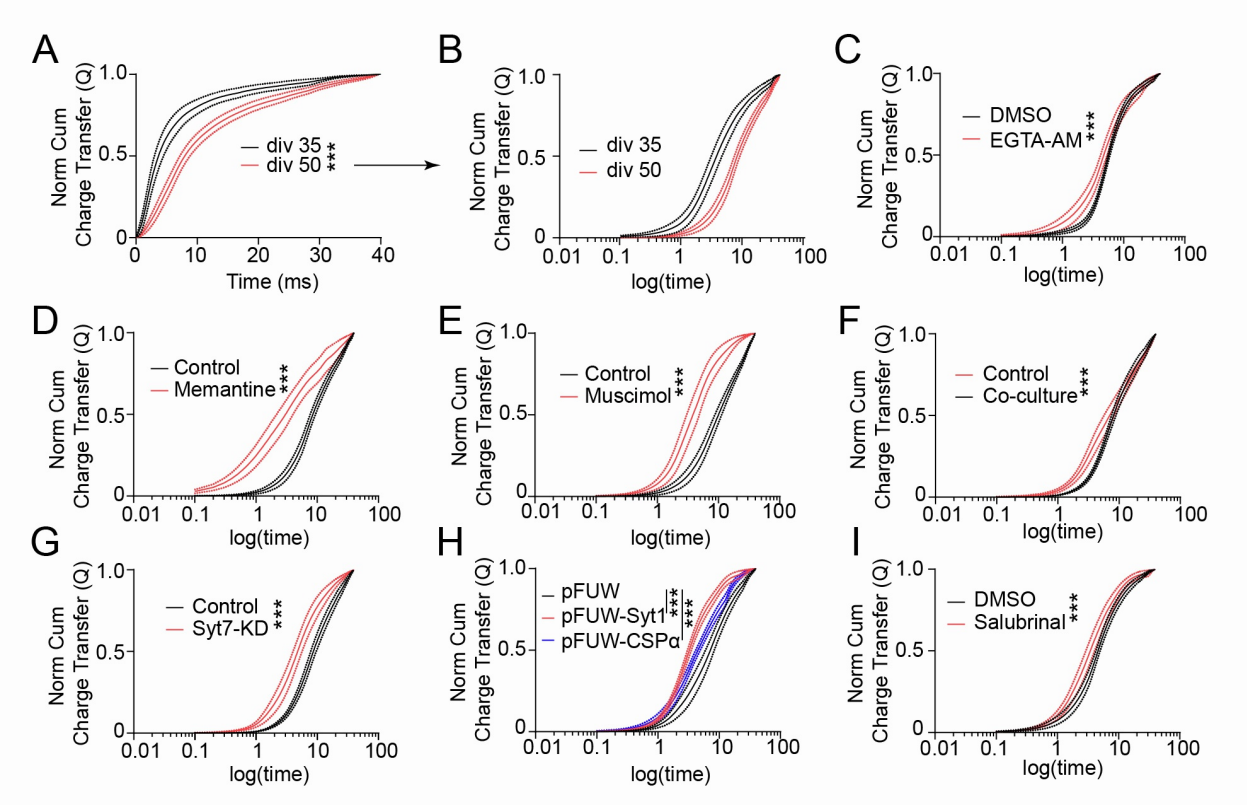
show mean±SEM ; eEPSC, n=9; eIPSC,n=12;N=1; p=0.0252; Mann Whitney U Test. Representative trace of an eIPSC that disappears following bicuculline perfusion.



Supplemental Figure S4. Correlation Between Human Postmortem Cortical Protein Levels and the Postmortem Interval, Related to Figure 7

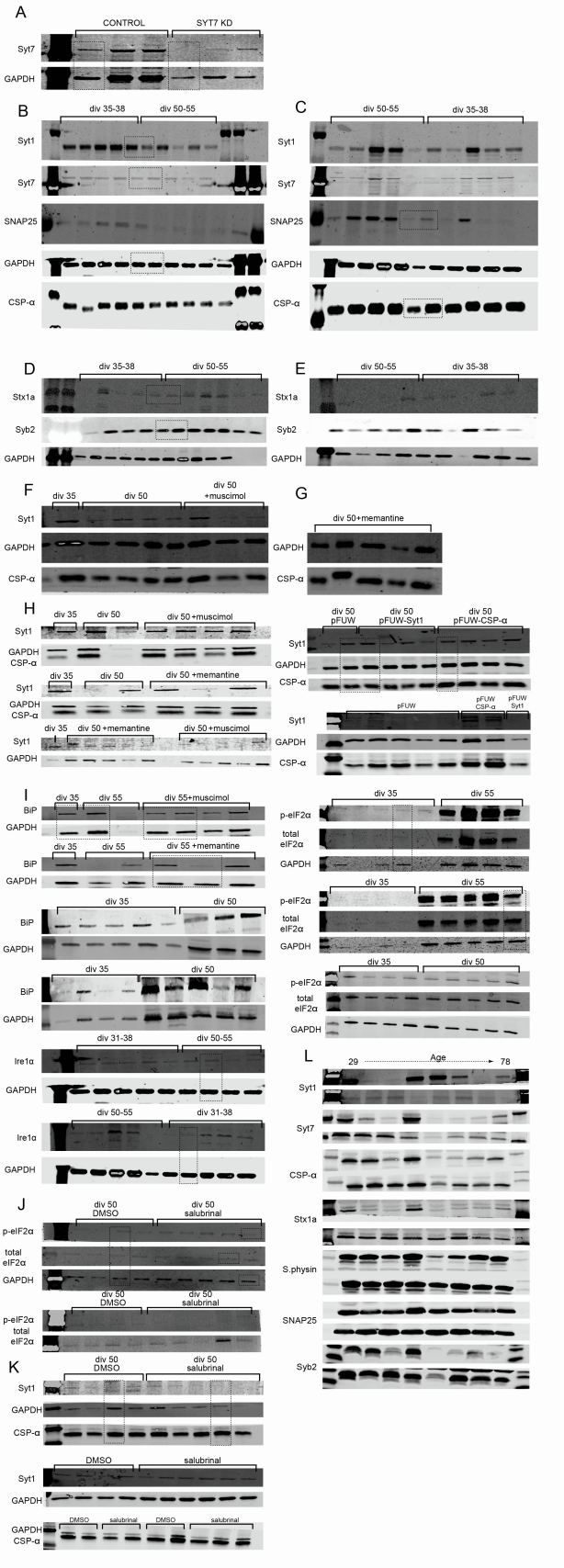
(A-F) Linear regression analysis shows that postmortem interval is not significantly correlated with protein levels; n=3 per subject, out of 8 subjects; bars show mean±SEM; p=0.611

(Syt1); Simple Linear Regression; p=0.138 (CSP-α); Simple Linear Regression; p= 0.809 (Stx1a); Simple Linear Regression; p=0.988 (Syb2); Simple Linear Regression; p=0.194 (SNAP25); Simple Linear Regression; p=0.16 (Syt7); Simple Linear Regression.



Supplemental Figure S5. Analysis of eEPSC Normalized Cumulative Charge Transfer, up to 40 milliseconds following stimulation, Related to STAR Methods

(A-I) Comparison of normalized cumulative charge transfer 40 milliseconds following stimulation. Starting in S5B, the x-axis is shown in logarithmic scale to underscore the robust differences in release synchrony; bars show mean±SEM; div 35,n=8, div 50,n=10; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test; DMSO, n=10, EGTA-AM, n=11; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test Control, n=12, Memantine, n=10; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test Control, n=10, Muscimol, n=7; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test Control, n=6, Co-culture, n=9; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test Control, n=7, Syt7-KD, n=12; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test; pFUW, n=12, pFUW-Syt1, n=12, pFUW-CSPα, n=11; N=2; p<0.001, Simple Linear Regression; p<0.0001, Kolmogorov Smirnov Test for both pFUW vs pFUW –Syt1 and pFUW vs pFUW-CSPα; DMSO, n=13, Salubrinal, n=15 N=3; p<0.001, Simple Linear Regression; p=0.0001, Kolmogorov Smirnov Test.


A
CONTROL
SYT7 KD
Syt7
GAPDH
B
div 35-38
div 50-55
Syt1
Syt7
SNAP25
GAPDH
CSP-α
C
div 50-55
div 35-38
Syt1
Syt7
SNAP25
GAPDH
CSP-α
D
div 35-38
div 50-55
Stx1a
Syb2
GAPDH
E
div 50-55
div 35-38
Stx1a
Syb2
GAPDH
F
div 35
div 50
div 50 +muscimol
Syt1
GAPDH
CSP-α
G
div 50+memantine
GAPDH
CSP-α
H
div 35
div 50
div 50 +muscimol
Syt1
GAPDH
CSP-α
div 35
div 50
div 50 +memantine
Syt1
GAPDH
CSP-α
div 35
div 50 +memantine
div 50 +muscimol
Syt1
GAPDH
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pFUW
div 50
pFUW-Syt1
div 50
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Syt1
GAPDH
CSP-α
pFUW
pFUW
CSP-α
pFUW
Syt1
Syt1
GAPDH
CSP-α
I
div 35
div 55
div 55+muscimol
BiP
GAPDH
div 35
div 55
div 55 +memantine
BiP
GAPDH
div 35
div 50
BiP
GAPDH
div 35
div 50
BiP
GAPDH
div 31-38
div 50-55
Ire1α
GAPDH
div 50-55
div 31-38
Ire1α
GAPDH
div 35
div 55
p-eIF2α
total eIF2α
GAPDH
div 35
div 55
p-eIF2α
total eIF2α
GAPDH
div 35
div 50
p-eIF2α
total eIF2α
GAPDH
J
div 50
DMSO
div 50
salubrinal
p-eIF2α
total eIF2α
GAPDH
div 50
DMSO
div 50
salubrinal
p-eIF2α
total eIF2α
K
div 50
DMSO
div 50
salubrinal
Syt1
GAPDH
CSP-α
DMSO
salubrinal
Syt1
GAPDH
DMSO
salubrinal
DMSO
salubrinal
GAPDH
CSP-α
L
29
Age
78
Syt1
Syt7
CSP-α
Stx1a
S.physin
SNAP25
Syb2

Supplemental Figure S6. The Raw Western Blotting Images, Related to STAR Methods. *Blot images are presented in grayscale. The representative images used in the main figures are indicated with dashed rectangles.*

(A) The raw western blot image showing Syt-7 and GAPDH bands of control and Syt-7 KD groups.

(B-E) The raw western blot images showing Syt-1, Syt-7,SNAP25, CSP-α, Stx1a, Syb2 and GAPDH bands of iN cells at div 35-38 to div 50-55.

(F-H) The raw western blot images showing Syt-1, CSP-α and GAPDH bands of iN cells at div 35, div 50 and div 50 upon either muscimol or memantine treatment and div 50s upon infection with lentivirus containing control, Syt-1 or CSP-α constructs.

(I-J) The raw western blot images showing BiP, Ire1α, p-eIF2α, total eIF2α and GAPDH bands of iN cells at div 35, div 50 and div 50 upon either muscimol, memantine, salubrinal or DMSO treatment.

(K) The raw western blot images showing Syt-1, CSP-α and GAPDH bands of iN cells at div 50 upon either salubrinal or DMSO treatment.

(L) The raw western blot images showing Syt-1, Syt-7,SNAP25, CSP-α, Stx1a, Syb2 and Synaptophysin bands of human postmortem cortical samples (n=2 replicates out of 3; for the other replicate please see Figure 7) *For consistency, different synaptic proteins were blotted on the same membrane, and the bands indicating different proteins were analyzed based on their corresponding molecular weights.*

Sample #	DIAGNOSIS	AGE	PMI	SEX	HEMISPHERE
1	Control	29	18.2	M	L
2	Control	54	24.2	M	L
3	Control	62	16.4	F	L
4	Control	67	22.3	M	R
5	Control	70	22.5	F	L
6	Control	74	12.5	F	L
7	Control	78	14.1	F	R
8	Control	78	23.9	F	R

Supplemental Table S2. Demographics of Human Postmortem Cortical Donors, Related to Figure 7. *(PMI: Postmortem Interval, hours)*