FIGURE LEGENDS

Figure 1

Detergent-insoluble EAAT2 is present in AD frontal cortex. Liquid chromatography tandem mass spectrometry (LC-MS-MS) was performed on total Triton X-100-insoluble proteins pooled from cortex of five AD patients. **(A)** A total of 348 EAAT2 tryptic fragments were identified that corresponded to the 24 unique EAAT2 sequences shown. **(B)** These EAAT2 tryptic fragments mapped to six primary structural regions indicated by gray highlighting that were located throughout the molecule. The EAAT2 model shown was modified from x-ray crystal structure results of Yernool and colleagues (2004). The primary transmembrane domains are indicated (I-VIII). Hairpin structures denoted as HP1 and HP2 form the glutamate-binding/gating domain.

Figure 2

Specificity of EAAT2 detection. Two different EAAT2 antibodies (AB12 and GLT-1A) were used that recognize the N- and C-terminus, respectively. Pan-specific AB12 recognizes all EAAT2/GLT-1 isoforms and GLT-1A recognizes EAAT2a/GLT-1a, the most abundant EAAT2 isoform in cortex and hippocampus. Using brain tissue from GLT-1 wild-type (WT) and knock-out (KO) mice **(A)**, ELISAs **(B)**, immunohistochemistry in cortex, and **(C)** western blots prove the specificity of EAAT2/GLT-1 detection. Functionally, EAAT2/GLT-1 is a homo-trimer that resolves as monomers (m), dimers (d), and apparent trimers (t) with approximate molecular weights of 70 kDa, 150 kDa, and >200 kDa, respectively. Error bars in panel A indicate standard error of the mean (SEM) of WT and KO brain tissue samples measured in triplicate.

Figure 3

In hippocampus detergent-insoluble EAAT2 levels were increased in patients with AD pathology. (A) Total Triton X–100-insoluble proteins from Parkinson's disease (PD, N=4), Normal control (Norm, N=13), CDR=0.5 (N=14), and later stage Alzheimer's disease (AD, N=22) patients were solublized with formic acid and analyzed by AB12 ELISAs to quantify detergent-insoluble EAAT2 levels. **(B)** Total Triton X-100-insoluble EAAT2 levels were measured by GLT-1A ELISAs as in panel A. **(C)** Scatter plot shows correlation between insoluble EAAT2 levels measured by AB12 and GLT-1A ELISAs for all subjects (N=53). P value indicates statistical significance determined by Pearson's correlation. **(D)** Total Triton X-100-insoluble levels of PS1 were measured in same samples as panels A and B. **(E, F)** Triton X-100-soluble EAAT2 levels from same samples as in panels A, B, and D were measured by AB12 and GLT-1A ELISAs, respectively. P values in panels A, B, D, E, and F indicate results of overall single-factor ANOVAs.

Figure 4

In frontal cortex detergent-insoluble EAAT2 levels were increased in patients with AD pathology. (A) Total Triton X–100-insoluble proteins from Parkinson's disease (PD, N=4), Normal control (Norm, N=20), CDR=0.5, (N=23), and later stage Alzheimer's disease (AD, N=55) patients were solublized with formic acid and analyzed by AB12 ELISA to quantify detergent-insoluble EAAT2 levels. **(B)** Total Triton X-100-insoluble EAAT2 levels were measured by GLT-1A ELISAs as in panel A. **(C)** Scatter plot shows correlation between insoluble EAAT2 levels measured by AB12 and GLT-1A ELISAs for all subjects (N=102). P value indicates statistical significance determined by Pearson's correlation. **(D)** Total Triton X-100-insoluble levels of PS1 were measured in same samples as panels A and B. **(E, F)** Triton X-100-soluble EAAT2 levels from same samples as in panels A, B, and D were measured by

AB12 and GLT-1A ELISAs, respectively. P values in panels A, B, D, E, and F indicate results of overall single factor ANOVAs.

Figure 5

EAAT2 immunohistochemistry in AD and normal controls. (A) EAAT2 immunostained with AB12 (brown) in AD frontal cortex revealed an irregular patchy astrocyte-like expression pattern that did not correspond with senile plaques immunostained red with the monoclonal antibody 4G8, which recognizes Aβ. Panel **(B)** is a higher magnification of the boxed region indicated in panel A and highlights the lack of correspondence between EAAT2 expression and amyloid deposits. Some plaques appeared to be surrounded by EAAT2 immunoreactivity, while other plaques were localized in EAAT2 immuno-negative domains. Patchy EAAT2 staining was observed in both AD and normal control cortex (not shown) and thus was not a distinctive feature of EAAT2 expression in AD. **(C and D)** EAAT2 was immunostained with AB12 (brown) in the hippocampal CA1 region of an AD (panel C) and a normal control (panel D). EAAT2 expression was restricted primarily to densely stained peri- and extra-synaptic EAAT2-positive puncta in the neuropil. **(E)** Example of apparent astrocyte immunostained with AB12 in AD frontal cortex where proximal and distal glial processes, as well the cell body displayed EAAT2 immunoreactivity. Prominent EAAT2 immunostaining in astrocyte-like cell bodies was more readily detected in AD brains than in controls. **(F)** AB12 immunostained apparent astrocytes in normal control revealed abundant EAAT2 immunoreactivity in proximal and distal processes, with less prominent cell body staining.

TABLE 1

Group	N	M:F	Age (years)	PMI (hours)	CERAD Score	Braak Stage
Hippocampus						
AD	22	6:5	84.3 +/- 2.2	11.4 +/- 1.4	Frequent (moderate-frequent)	VI (V-VI)
CDR=0.5	14	7:8	88.9 +/- 2.2	13.0 +/- 2.0	Sparse (none-frequent)	IV (I-VI)
Norm	13	8:5	87.0 +/- 1.7	9.8 +/- 1.3	Sparse (none-sparse)	II (I-III)
PD	4	1:1	81.0 +/- 5.7	17.8 +/- 2.2	Sparse (none-sparse)	II (II-III)
Frontal Cortex						
AD	55	6:5	82.7 +/- 1.3	11.6 +/- 0.8	Frequent (sparse-frequent)	VI (V-VI)
CDR=0.5	23	1:1	90.4 +/- 1.8	12.5 +/- 1.4	Sparse (none-frequent)	III (I-VI)
Norm	20	2:3	90.1 +/- 1.7	11.2 +/- 1.2	None (non-sparse)	II (I-III)
PD	4	3:1	84.9 +/- 3.7	10.5 +/- 4.3	None (none-sparse)	II (I-III)

Patient Demographic and Pathologic Characteristics: In the hippocampus panel, Consortium to Establish a Registry for Alzheimer's Disease (CERAD) scores and Braak stage, but not age at death (Age) were significantly higher for the later stage AD group (Fisher's Exact: [9]=46.385, $p<0.00001$ and [18]=71.067, $p<0.00001$, respectively). In both the hippocampus and frontal cortex panels, the male/female (M:F) ratio and postmortem interval (PMI) were statistically equivalent. In the frontal cortex panel later stage AD patients tended to die earlier than other subjects ($F[3,98]=5.708$, $p<0.001$) and had significantly higher CERAD scores and Braak staging (Fisher's exact: [9]=87.238, $p<0.00001$; [18]=115.049, $p<0.00001$, respectively). Age and PMI are indicated as means +/- standard error of mean (SEM). CERAD scores and Braak Stages are indicated as medians with the range note in parentheses. Abbreviations: Number of subjects (N), Males: Females (M:F), post mortem interval (PMI),

Clinical Dementia Rating (CDR), Consortium to Establish a Registry for Alzheimer's Disease (CERAD), Alzheimer's disease (AD), Normal controls (Norm), Parkinson's disease (PD).

A

CLEENLGIDK
DGMNVLGLIGFFIAFGIAMGK
DLGWAR
ESNSNQCVYAAINSVIVIDCK
GLEPK
HLGLR
IIAIK
LASPINPDVYMLIAFPGDILMR
LMVDFFNILNEIVMK
LVIHIMWYSPLGIACLICGK
MASTDGAMNNPK
MGDQAK
MRDSHLGSEEPK
NLILPLIISSLITGLSGLDAK
NDEVSSLDAFLDLIR
NLFPENLVQACPQQIQTVTK
NLLLTLTVFGVILGAVCGGLLR
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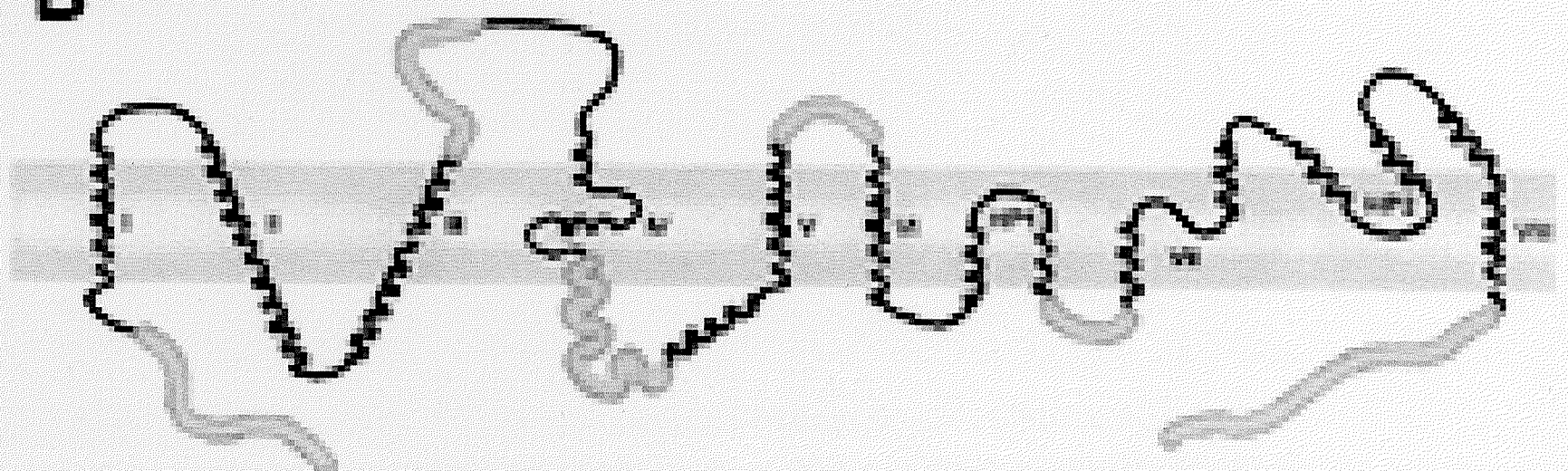
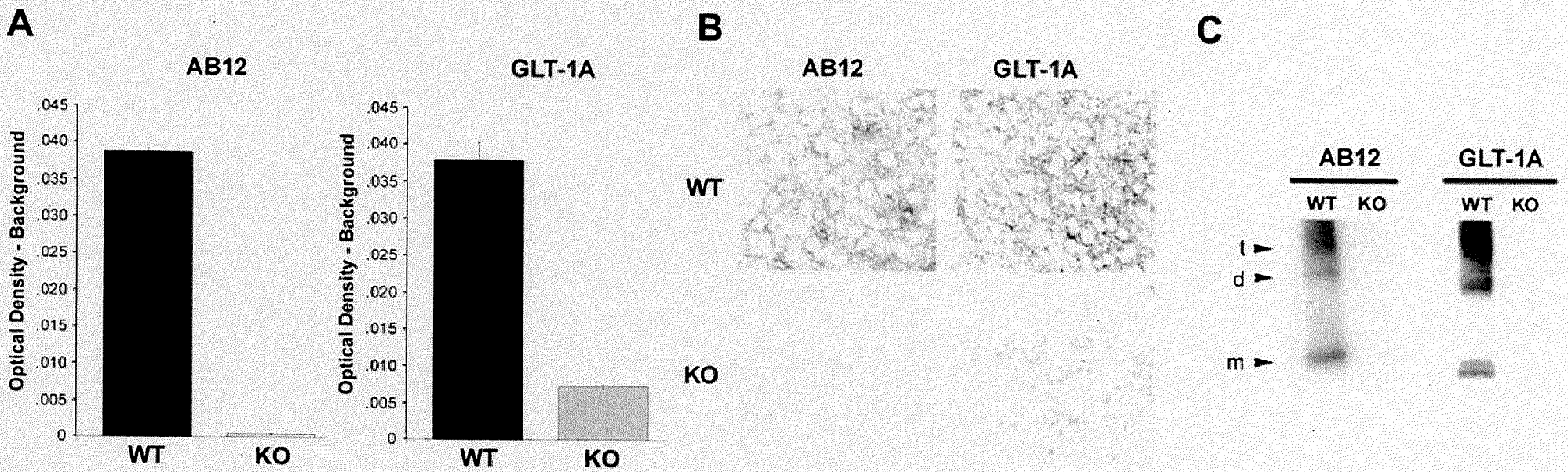
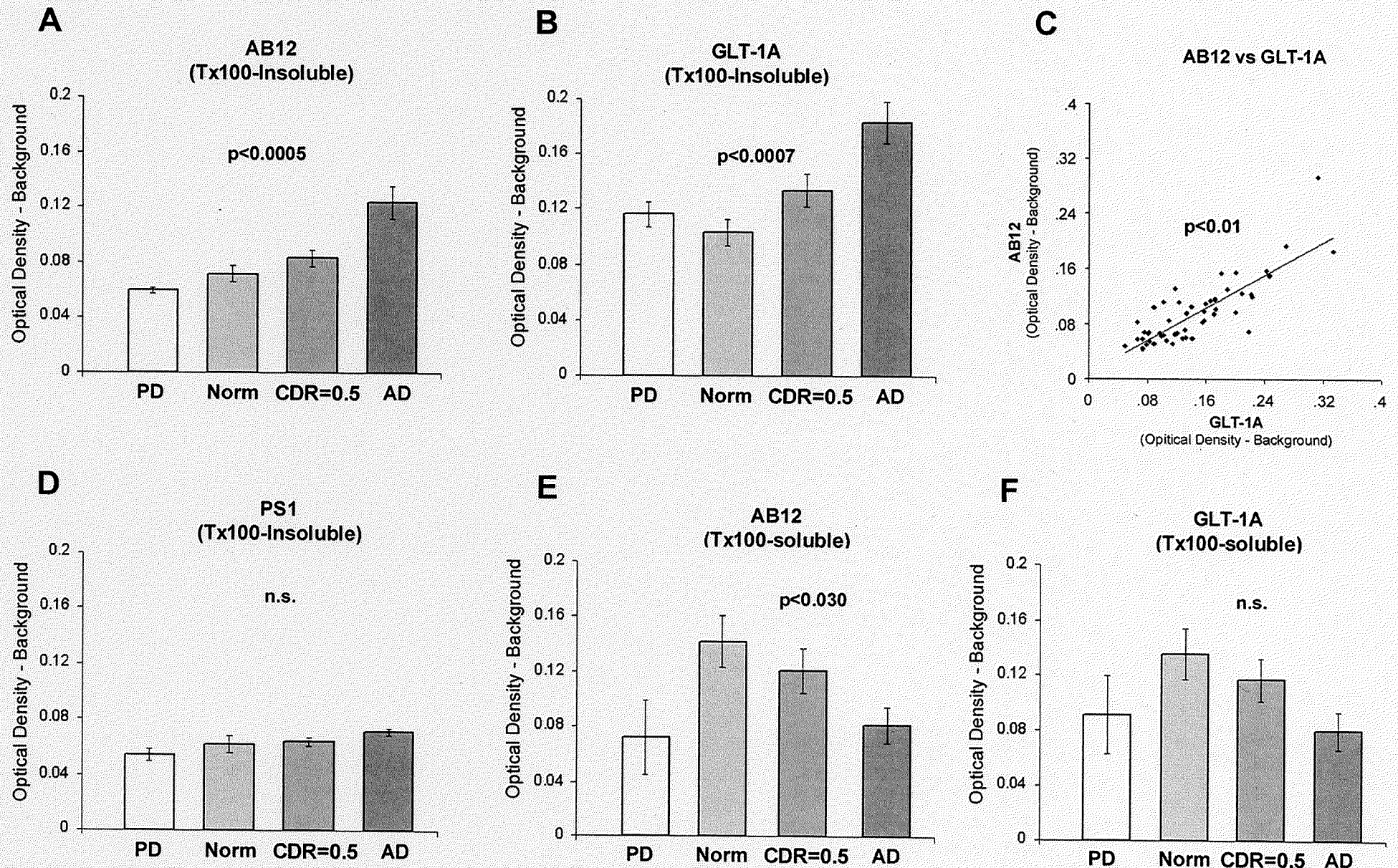
VMEDIEMTK
VTLAANGK

B




A
AB12
GLT-1A
Optical Density - Background
.045
.040
.035
.030
.025
.020
.015
.010
.005
0
WT
KO
B
AB12
GLT-1A
WT
KO
C
AB12
GLT-1A
WT
KO
WT
KO
t
d
m


A
AB12
(Tx100-Insoluble)
Optical Density - Background
0.2
0.16
0.12
0.08
0.04
0
p<0.0005
PD
Norm
CDR=0.5
AD
B
GLT-1A
(Tx100-Insoluble)
Optical Density - Background
0.2
0.16
0.12
0.08
0.04
0
p<0.0007
PD
Norm
CDR=0.5
AD
C
AB12 vs GLT-1A
AB12
(Optical Density - Background)
.4
.32
.24
.16
.08
0
p<0.01
0
.08
.16
.24
.32
.4
GLT-1A
(Optical Density - Background)
D
PS1
(Tx100-Insoluble)
Optical Density - Background
0.2
0.16
0.12
0.08
0.04
0
n.s.
PD
Norm
CDR=0.5
AD
E
AB12
(Tx100-soluble)
Optical Density - Background
0.2
0.16
0.12
0.08
0.04
0
p<0.030
PD
Norm
CDR=0.5
AD
F
GLT-1A
(Tx100-soluble)
Optical Density - Background
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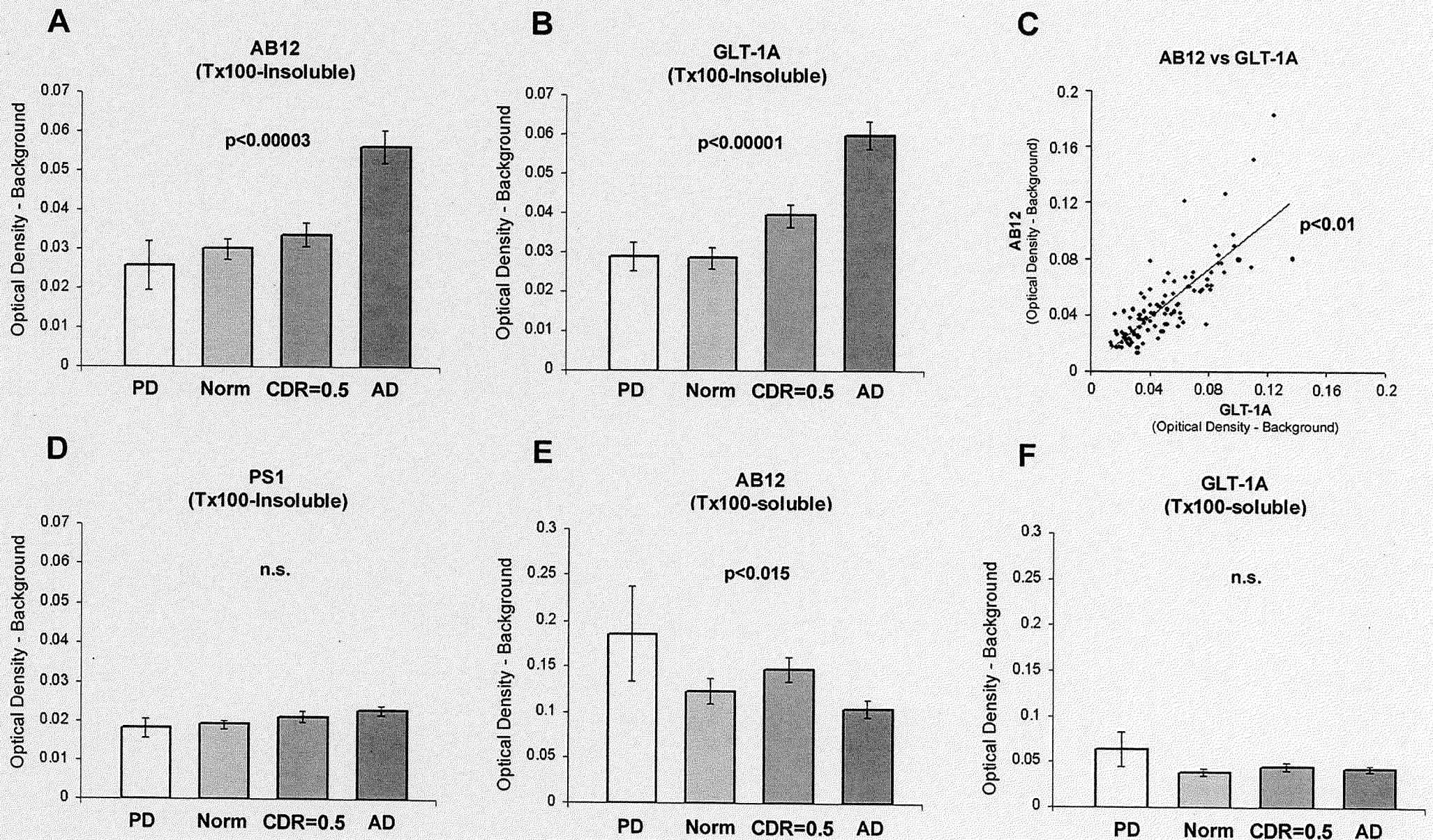
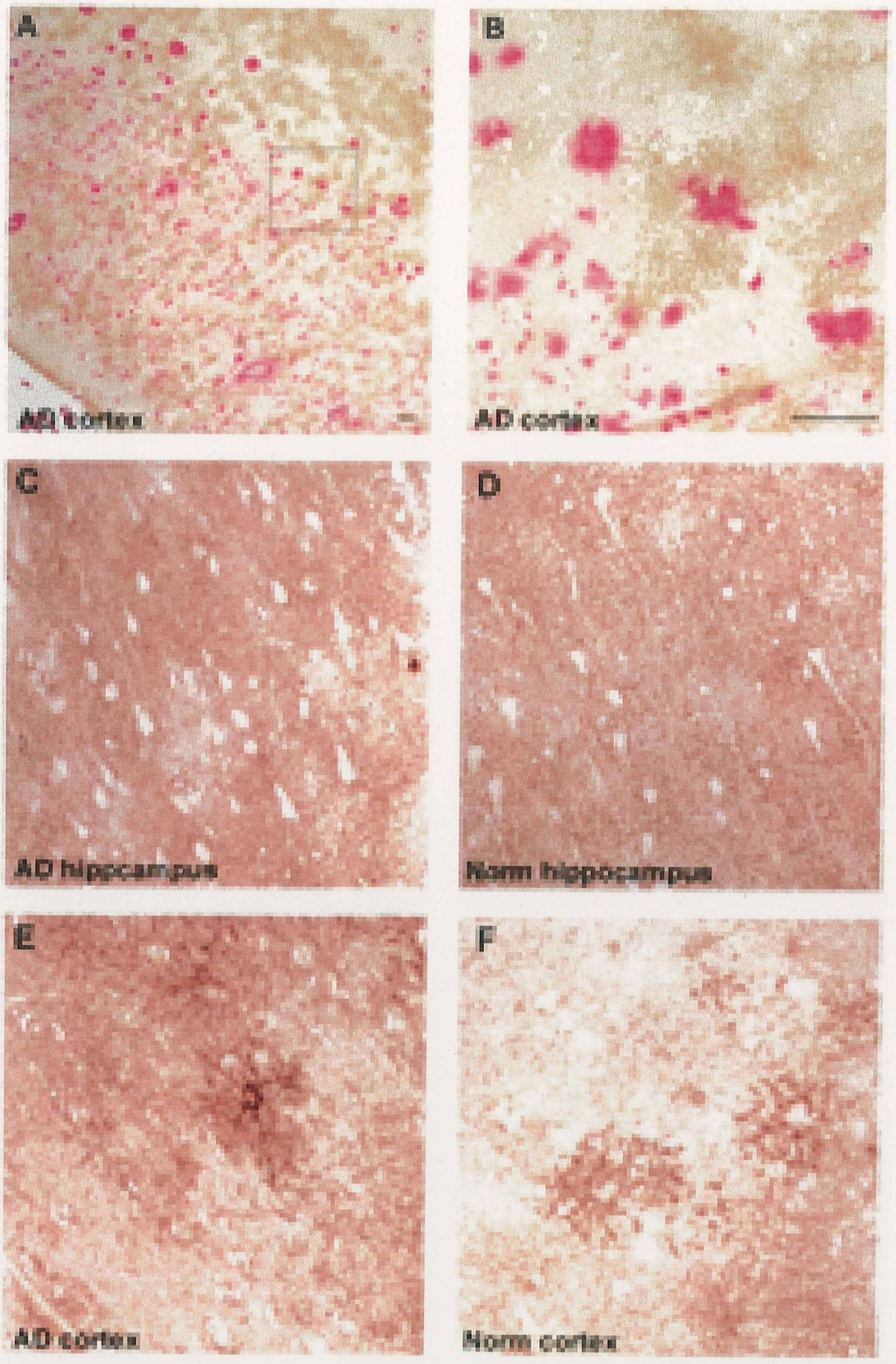
0.16
0.12
0.08
0.04
0
n.s.
PD
Norm
CDR=0.5
AD


A
AB12
(Tx100-Insoluble)
p<0.00003
Optical Density - Background
PD
Norm
CDR=0.5
AD
B
GLT-1A
(Tx100-Insoluble)
p<0.00001
C
AB12 vs GLT-1A
p<0.01
AB12
(Optical Density - Background)
GLT-1A
(Opitical Density - Background)
D
PS1
(Tx100-Insoluble)
n.s.
E
AB12
(Tx100-soluble)
p<0.015
F
GLT-1A
(Tx100-soluble)
n.s.


A
AD cortex
B
AD cortex
C
AD hippcampus
D
Norm hippocampus
E
AD cortex
F
Norm cortex