



## Anti-GluA3 antibodies in frontotemporal dementia: effects on glutamatergic neurotransmission and synaptic failure



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### ABSTRACT

Despite the great effort of the scientific community in the field, the pathogenesis of frontotemporal dementia (FTD) remains elusive. Recently, a role for autoimmunity and altered glutamatergic neurotransmission in triggering disease onset has been put forward. We reported the presence of autoantibodies recognizing the GluA3 subunit of  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptors in about 25% of FTD cases. In this study, we evaluated the mechanisms involved in anti-GluA3 autoimmunity, through molecular/neurochemical analyses conducted on patients' brain specimens with frontotemporal lobar degeneration–tau neuropathology. We then corroborated these results *in vivo* in FTD patients with transcranial magnetic stimulation and glutamate, D-serine, and L-serine dosages in the cerebrospinal fluid and serum. We observed that GluA3 autoantibodies affect glutamatergic neurotransmission, decreasing glutamate release and altering GluA3-containing  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor levels. These alterations were accompanied by changes of scaffolding proteins involved in receptor synaptic retention/internalization. The above results were confirmed by transcranial magnetic stimulation, suggesting a significant impairment of indirect measures of glutamatergic neurotransmission in FTD patients compared with controls, with further add-on harmful effect in those FTD patients with anti-GluA3 antibodies. Finally, FTD patients showed a significant increase of glutamate, D-serine, and L-serine levels in the cerebrospinal fluid.

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### 1. Introduction

Frontotemporal lobar degeneration (FTLD), a common cause of presenile dementia, is a clinically and neuropathologically heterogeneous disorder. It is characterized by behavioral abnormalities, personality change, deficits of executive functions, and language impairment (Hodges and Piguet, 2018; Seelaar et al., 2011). The neuropathological substrate is complex. Hyperphosphorylated tau or transactive response DNA-binding protein

43 (TDP-43) are the most frequent underlying proteinopathies responsible for FTLD-tau or FTLD-TDP43, respectively (Mackenzie et al., 2010, 2011). Clinically, we refer to frontotemporal dementia (FTD) to encompass 3 clinical syndromes, namely the behavioral variant FTD (bvFTD) (Rascovsky et al., 2011), the agrammatic variant of primary progressive aphasia (avPPA), and the semantic variant of PPA (svPPA) (Gorno-Tempini et al., 2011).

Up to 40% of cases have a family history of dementia, with an autosomal dominant inheritance in around a third of patients (Benussi et al., 2015; Ferrari et al., 2014; Rohrer et al., 2015; Stevens et al., 1998). Mutations within microtubule-associated protein tau (MAPT) (Hutton et al., 1998), granulin (GRN) (Baker et al., 2006; Cruts et al., 2006), and chromosome 9 open reading frame 72 (C9orf72) (DeJesus-Hernandez et al., 2011; Renton et al., 2011) genes are proven major

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causes of genetic FTD, accounting for 10%–20% of all FTD cases (Rohrer et al., 2009).

In the last years, a number of evidence have suggested a new player in FTD pathogenesis, arguing for a role of autoimmunity in triggering disease onset (Alberici et al., 2018). This hypothesis stemmed from epidemiological data and clinical studies, reporting a significantly increased risk of autoimmune disorders (Miller et al., 2013, 2016) and autoimmune system dysregulation in FTD patients (Cavazzana et al., 2018), and from genetic research that argued for immune-mediated genetic enrichment in FTD, particularly within the human leukocyte antigen region (Broce et al., 2018; Ferrari et al., 2014). The identification of a dysregulation of the immune system in FTD might open new routes for therapeutic perspectives in autoimmune-related neurodegeneration to reduce or revert disease progression.

We have recently reported a high frequency of autoantibodies recognizing the GluA3 subunit of  $\alpha$ -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors in patients with FTD (Borroni et al., 2017), corroborating the role of glutamatergic neurotransmission in this disorder (Murley and Rowe, 2018). Furthermore, in both rat hippocampal neuronal primary cultures and in human neurons derived from induced pluripotent stem cells (iPSCs), we demonstrated that anti-GluA3 antibodies were detrimental for neurons and for AMPA function (Borroni et al., 2017). However, it is still unclear whether anti-AMPA receptor antibodies are the trigger event for protein misfolding or whether they are produced as a consequence of neuronal loss, boosting, in turn, the on-going neurodegenerative process. Moreover, it needs to be investigated whether anti-AMPA GluA3 receptor antibodies mediate a detrimental effect *in vivo* in FTD patients and how these antibodies modify presynaptic and postsynaptic glutamatergic neurotransmission.

To this, in the present work we used a multidisciplinary approach to unravel the role of glutamatergic transmission and anti-AMPA GluA3 receptor autoimmunity in autopsy of FTLD-tau patients and *in vivo* in FTD patients. In particular, a) we evaluated glutamate receptors composition at synapses in autopsied brain specimens from patients affected by FTLD, considering only FTLD-tau patients to have a more homogeneous sample; b) we investigated the impact of anti-GluA3 antibodies on the AMPA-mediated control of presynaptic glutamate release *in vivo* by using cerebrospinal fluid (CSF) obtained from patients positive for anti-GluA3 antibodies; c) we indirectly assessed *in vivo* glutamatergic neurotransmission integrity by transcranial magnetic stimulation (TMS) in both anti-GluA3 antibody-positive and anti-GluA3 antibody-negative FTD patients, compared with controls, and d) we measured L-glutamate and D-/L-serine levels in the CSF and serum of FTD patients with or without anti-GluA3 autoantibodies.

## 2. Materials and methods

### 2.1. Serum and CSF dosage of anti-GluA3 antibody levels

Serum samples were frozen immediately after centrifugation and stored at  $-80^{\circ}\text{C}$  before enzyme-linked immunosorbent assay. The detection of anti-GluA3 antibodies (peptide A and peptide B) was performed by enzyme-linked immunosorbent assay according to a previously published protocol (Borroni et al., 2017; Mantegazza et al., 2002).

CSF stored at  $-80^{\circ}\text{C}$  or in liquid nitrogen was used for anti-GluA3 antibody dosage. The same protocol used for serum was applied to test anti-GluA3 antibody peptide A in the CSF, except for plates (Immobilon 4HBX 96-well plates; Dynatech, Germany) and the working dilution (1:2 for CSF and 1:4500 for the secondary antibody) (Borroni et al., 2017).

### 2.2. Biochemical assays

The temporal ( $n = 6$ ) and occipital ( $n = 5$ ) cortex from FTLD-tau patients were obtained from The Netherlands Brain Bank, Netherlands Institute for Neuroscience, Amsterdam (open access: [www.brainbank.nl](http://www.brainbank.nl)). All material has been collected from donors for or from whom written informed consent, for research purpose, had been obtained by the Netherlands Brain Bank.

We considered patients with FTLD-tau to obtain a more homogeneous sample group based on our previous findings on the effect of anti-GluA3 antibodies on tau metabolism (Borroni et al., 2017). Western blot analysis was performed in the total homogenate and Triton-insoluble postsynaptic fractions. Briefly, brain specimens were manually homogenized twice in lysis buffer (Sucrose 0.32 M, Hepes 1 mM, magnesium chloride 1 mM, sodium carbonate 1 mM) and centrifuged at  $900 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . The resulting supernatants were pooled and centrifuged  $13,000 \times g$  for 15 minutes at  $4^{\circ}\text{C}$ . Pellets were then resuspended in 1 mM Hepes and ultra-centrifuged  $100,000 \times g$  for 1 hour at  $4^{\circ}\text{C}$ . Precipitates were dissolved, incubated for 15 minutes in 150 mM potassium chloride, 0.5% Triton, and ultra-centrifuged again  $100,000 \times g$  for 1 hour at  $4^{\circ}\text{C}$ . The final pellets (Triton-insoluble postsynaptic fractions) were homogenized with a glass-glass potter in Hepes 20 mM buffer. All purification steps were performed in the presence of protease and phosphatase inhibitor cocktails (Complete; Roche Diagnostics, Monza, Italy). After separation by SDS-PAGE on an 8% gel under denaturing conditions, the proteins were electrotransferred onto a nitrocellulose membrane. Membranes were blocked with I-block solution (Invitrogen, Thermo Scientific, Milan, Italy) and incubated overnight with primary antibodies (anti-GluA1 antibody, BK131855, 1:500; Cell Signaling; anti-GluA2 antibody, #75-002, 1:500; Neuromab; anti-GluA3 antibody, #182203, 1:1000; Synaptic System; anti-GluA1 p845, ab76321, 1:1000; Abcam; anti-GRIP1 antibody, Ab25963, 1:500; Abcam; anti-PICK1 antibody, Ab3420, 1:500; Abcam; anti-Tubulin antibody, T9026, 1:5000; Sigma Aldrich), followed by incubation with horseradish peroxidase-linked anti-rabbit or anti-mouse IgG antibody (1:5000; Bio-Rad, Hercules, CA, USA) in TBS containing 0.1% Tween-20 at room temperature for 1 h. Finally, proteins were visualized using an electrochemical luminescence (ECL) kit (Clarity Western ECL substrate; Bio-Rad or LiteAblot TURBO; EuroClone, Milan, Italy), and the images were obtained using ChemiDoc Imaging System (Bio-Rad). Quantification was performed using ImageJ software, and each protein was normalized on the corresponding Tubulin band.

### 2.3. Purification of synaptosomes and release experiments

Mice (male, strain C57BL/6J) were obtained from Charles River (Calco, Italy) and were housed in the animal facility of DIFAR, University of Genoa, under environmentally controlled conditions (ambient temperature =  $22^{\circ}\text{C}$ , humidity = 40%) on a 12-h light/dark cycle with food and water *ad libitum*. Mice were euthanized by cervical dislocation, and subsequently decapitated, and the hippocampi were rapidly removed. The experimental procedures followed the European legislation (European Communities Council Directive of 24 November 1986, 86/609/EEC) and the ARRIVE guidelines, and those were approved by the Italian Ministry of Health (DDL 26/2014 and previous legislation; protocol number n° 50/2011-B). Experiments were performed following the Guidelines for Animal Care and Use of the National Institutes of Health and in accordance with the Society's Policies on the Use of Animals and Humans in Neuroscience Research. In line with the 3Rs rules (replacement, refinement, and reduction), any effort was made to reduce the number of animals to obtain statistically reliable results. Synaptosomes were prepared by homogenizing the cortex as

previously described (Summa et al., 2011). Synaptosomes were resuspended in a physiological solution with the following composition (mM): NaCl, 140; KCl, 3; MgSO<sub>4</sub>, 1.2; CaCl<sub>2</sub>, 1.2; NaH<sub>2</sub>PO<sub>4</sub>, 1.2; NaHCO<sub>3</sub>, 5; HEPES, 10; glucose, 10; pH 7.2–7.4 and incubated at 37 °C for 30 minutes in the presence of the patients' sera (dilution 1:200 to 1:100). After 15 minutes, [<sup>3</sup>H]D-aspartate ([<sup>3</sup>H]D-ASP, (specific activity 11.3 Ci/mmol; Perkin Elmer Boston, MA, USA, f.c.: 50 nM) was added to the incubation suspension. Synaptosomes were layered on microporous filters at the bottom of parallel thermostated chambers in a superfusion system (Pittaluga, 2016). Synaptosomes were superfused at 0.5 mL/min for 36 minutes with a standard physiological solution to equilibrate the system, and then, for 3 minutes, fractions were collected for monitoring tritium release. At t = 39 minutes of superfusion, synaptosomes were exposed to 50 μM D-AMPA (Tocris Bioscience, Bristol, UK) till the end of superfusion.

#### 2.4. FTD study population for TMS assessment and cerebrospinal fluid or serum analyses

For all participants, informed consent was obtained and sampling protocols were approved by the Ethics Committee of Brescia Hospital, Italy. The study was conducted in accordance with Helsinki Declaration.

Each included patient fulfilled current clinical criteria for FTD (Gorno-Tempini et al., 2011; Rascovsky et al., 2011). All patients considered in the present study underwent an extensive neuropsychological evaluation and brain magnetic resonance imaging. In a subgroup of patients, diagnosis was accomplished by amyloid markers such as cerebrospinal fluid Aβ<sub>42</sub> determinations or amyloid positron emission tomography imaging to rule out Alzheimer's disease, as previously published (Benussi et al., 2017).

Extra exclusion criteria were considered for patients undergoing TMS procedure: i) use of drugs that could affect TMS variables; ii) history of head trauma, alcohol abuse, stroke or transient ischemic attack, or epilepsy; and iii) presence of pacemaker or other cardiac devices, cochlear implants, or previous brain surgery, such as clipping of a cerebral aneurysm.

A group of healthy controls was included, who underwent a brief standardized neuropsychological assessment (Mini-Mental State Examination score ≥ 27/30); psychiatric or other neurological illnesses were considered as an exclusion criterion.

#### 2.5. Glutamatergic neurotransmission assessment in vivo in FTD patients by TMS

TMS was performed with a figure-of-eight coil (each loop diameter, 70 mm) connected to a Magstim BiStim<sup>2</sup> system (Magstim Company, Oxford, UK). The magnetic stimuli had a monophasic current waveform (rise time of 100 μs, decaying back to zero over 800 μs). Motor-evoked potentials (MEPs) were recorded from the right first dorsal interosseous muscle through surface Ag/AgCl electrodes placed in a belly-tendon montage and acquired using a Biopac MP-150 electromyograph (BIOPAC Systems Inc, Santa Barbara, CA). The TMS coil was held tangentially over the scalp region corresponding to the primary hand motor area contralateral to the target muscle, with the coil handle pointed 45° posteriorly and laterally to the sagittal plane. The motor hotspot was defined as the location where TMS consistently produced the largest MEP size at 120% of the resting motor threshold (rMT) in the target muscle and was marked with a felt-tip pen on the scalp to ensure stable placement of the coil throughout the experiment. rMT was defined as the minimal stimulus intensity needed to produce MEPs with an amplitude of at least 50 μV in 5 of 10 consecutive trials during complete muscle relaxation, which was controlled by visually

checking the absence of electromyography activity at high-gain amplification, as previously published (Benussi et al., 2016). Intracortical facilitation (ICF), which has been shown to be likely mediated by glutamatergic receptors, was studied at rest with a paired-pulse paradigm, delivered in a conditioning-test design, as previously reported. Briefly, the conditioning stimulus (CS) was set at an intensity of 70% of the rMT, whereas the test stimulus (TS) was adjusted to evoke a MEP approximately 1 mV peak-to-peak in the relaxed first dorsal interosseous. An interstimulus interval (ISI) of 7 ms between the CS and TS was applied (Ziemann and RothwellRidding, 1996). Ten stimuli were delivered for each ISI, and 14 control MEPs in response to the TS alone were recorded in all participants.

#### 2.6. HPLC detection of D-/L-serine and L-glutamate levels

CSF and serum sample collection from FTD patients and healthy controls were obtained at fasting (between 8.00 and 10.00 am) according to standard procedures. CSF was collected in sterile polypropylene tubes and gently mixed to avoid gradient effects. Sample size and demographic information are shown in Table 2. CSF and serum samples were analyzed as previously reported (Nuzzo et al., 2019). Briefly, 100 μL CSF or serum samples were mixed in a 1:10 dilution with high performance liquid chromatography (HPLC)-grade methanol (900 μL) and centrifuged at 13,000 × g for 10 minutes. Supernatants were dried, suspended in 0.2 M trichloroacetic acid, and then neutralized with NaOH. Samples were then subjected to pre-column derivatization with o-phthalaldialdehyde/N-acetyl-L-cysteine in 50% methanol. Diastereoisomer derivatives were resolved on a Symmetry C8 5-μm reversed-phase column (Waters; 4.6 × 250 mm) in isocratic conditions (0.1 M sodium acetate buffer, pH 6.2, 1% tetrahydrofuran, 1 mL/min flow rate). A washing step in 0.1 M sodium acetate buffer, 3% tetrahydrofuran, and 47% acetonitrile was performed after every single run. Identification and quantification of D-Ser, L-Ser, and L-Glu was based on retention times and peak areas, compared with those associated with external standards. The amino acid level was expressed as μM, whereas the D-/total serine ratio was expressed as percentage (%).

#### 2.7. Statistical analysis

For HPLC experiments, identification and quantification of D-Ser, L-Ser, and L-Glu were based on retention times and peak areas, compared with those associated with external standards. The amino acid level was expressed as μM, whereas the D-/total serine ratio was expressed as percentage (%). Statistical analyses were performed by the Mann-Whitney or Kruskal-Wallis test followed by Dunn's test, when required. For release experiments the amount of radioactivity released into each superfuse fraction was expressed as fractional efflux. The AMPA-evoked release of tritium was quantified as percent increase over basal release. Analysis of variance was performed by ANOVA followed by Dunnett's multiple-comparisons test. The level of significance was set at p < 0.05. For Western blot experiments, significance of the differences was determined by unpaired Student's t-test. For TMS experiments, a one-way ANCOVA was run to determine the difference in intracortical connectivity between groups, corrected for disease severity (using the FTLD-modified Clinical Dementia Rating Scale). When a significant main effect was reached, post hoc tests with Bonferroni correction for multiple comparisons were conducted to analyze individual group differences. Statistical significance was assumed at p < 0.05. Data analyses were carried out using the SPSS 21.0 software.

**Table 1**

Demographic and clinical characteristics of autopsy FTLD-tau patients

Patient	GluA3_Ab+					GluA3_Ab-				
	1	2	3	4	5	6	7	8	9	10
Age at death, (y)	60	66	71	53	63	52	64	46	54	49
Age at onset, (y)	48	57	62	42	55	46	59	39	52	39
Disease duration, (y)	12	9	9	11	8	6	5	7	2	10
Gender	M	F	F	M	F	M	F	M	F	M
Onset symptom	Ex. dysf.	n.a.	Behavior	Memory	Personality changes	Memory	Behavior	Personality changes	n.a.	Personality changes
Clinical diagnosis	AD	Pick's	Pick's	AD	Pick's	AD	Pick's	Pick's	Pick's	Pick's
CSF GluA3 autoantibodies titer (OD)	0.032 <sup>a</sup>	0.510	0.041	0.022 <sup>a</sup>	0.241 <sup>a</sup>	0.009	0.001	0.001 <sup>a</sup>	0.001 <sup>a</sup>	0.001 <sup>a</sup>
PMD	280	340	345	275	255	400	310	335	450	310
pH liquor	6,45	6,48	6,44	6,75	6,36	6,61	6,4	6,48	6,46	6,54

Key: GluA3\_Ab+, patients with GluA3 autoantibodies; GluA3\_Ab-, patients without GluA3 autoantibodies; M, male; F, female; n.a., not available; Ex. dysf., executive dysfunction; behavior, behavioral abnormalities; AD, Alzheimer's disease; CSF, cerebrospinal fluid; OD, optical density (internal cut-off >0.019); PMD, postmortem delay (minutes); FTLD, frontotemporal lobar degeneration.

<sup>a</sup> Used on autopsy specimens of the temporal and occipital cortex (see text for details).

## 2.8. Data availability

The experimental data that support the findings of this study are available from the corresponding author, upon reasonable request.

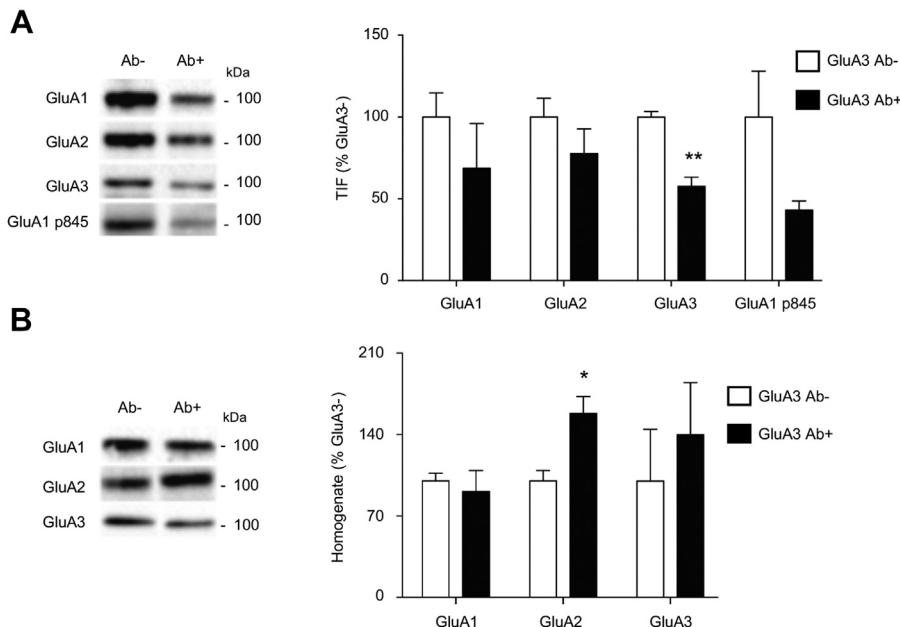
## 3. Results

### 3.1. Effect of GluA3 antibody on AMPA receptor subunit composition in the temporal cortex of FTLD-tau patients

Postmortem specimens of the temporal and occipital cortex of FTLD-tau patients were analyzed to evaluate the effects induced by GluA3 autoantibodies on the glutamatergic synapse. To this, we firstly analyzed 10 CSF samples for which autopsy was available, and we detected the presence of anti-GluA3 antibodies in 5 of 10 samples (see Table 1). Among these, we then considered

postmortem specimens from 3 FTLD-tau patients with CSF anti-GluA3 antibodies and 3 FTLD-tau patients without CSF anti-GluA3 antibodies for further analyses.

We previously reported that administration of CSF containing anti-GluA3 antibodies results in a significant decrease of the GluA3 subunit levels at postsynaptic sites both in rat primary hippocampal neurons and in cortical neurons obtained from human iPSCs (Borroni et al., 2017). Similarly, here we found that FTLD-tau GluA3\_Ab+ patients showed a significant reduction in GluA3 levels in a postsynaptic fraction purified from the temporal cortex (\*\* $p = 0.0038$ , unpaired Student's t-test, vs. GluA3\_Ab-, Fig. 1A), whereas there was no difference in the postsynaptic levels of GluA1 ( $p = 0.3702$ , unpaired Student's t-test, vs. GluA3\_Ab-, Fig. 1A), GluA2 ( $p = 0.3042$ , unpaired Student's t-test, vs. GluA3\_Ab-, Fig. 1A) AMPA receptor subunits and in the phosphorylated form of GluA1 (Ser845,  $p = 0.1297$ , unpaired Student's t-test, vs. GluA3\_Ab-,



**Fig. 1.** Neurobiological effect of GluA3 antibody on AMPA receptor's composition in the temporal cortex of FTLD-tau patients. Western blot quantification of GluA1, GluA2, GluA3, and phosphorylated ser845 GluA1 subunits in Triton-insoluble postsynaptic fractions (TIF) (A) and total homogenate (B) obtained from the temporal cortex of FTLD-tau patients with (Ab+, closed bars) or without (Ab-, open bars) anti-GluA3 antibodies. Left panel: representative blot; right panel: densitometric quantification. Tubulin was used for normalization. \* $p < 0.05$ , \*\* $p < 0.01$ , unpaired Student's t-test (mean of 3 experiments with  $n = 3$ ).

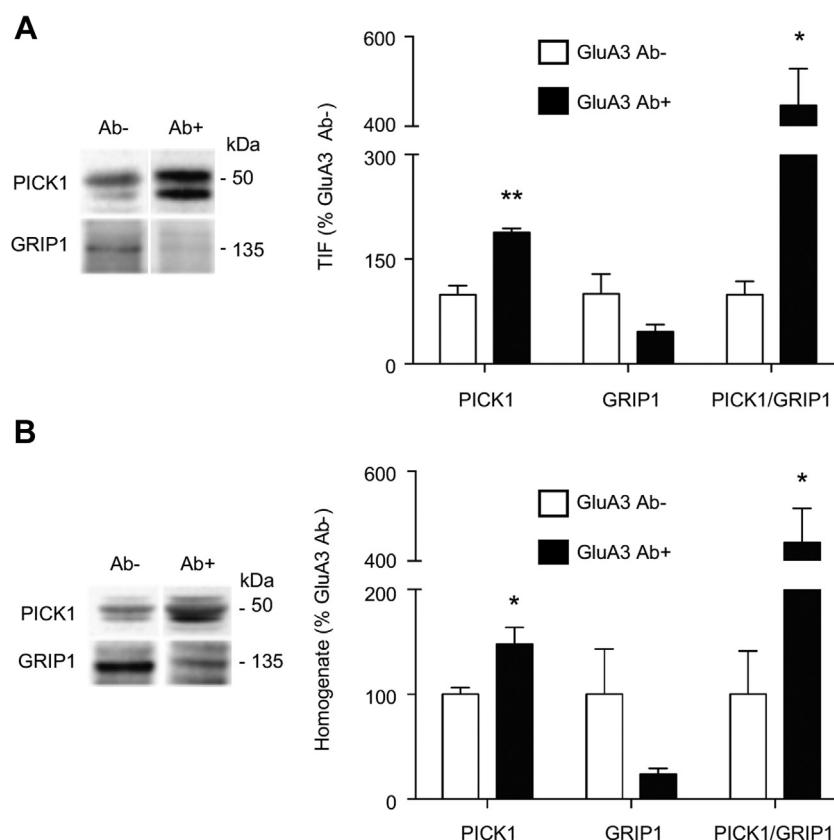
**Fig. 1A**), a well-validated marker of synaptic plasticity (Marcello et al., 2013; Oh et al., 2006; Song et al., 2016). Conversely, Western blotting performed in the total homogenate reveals a significant increase in GluA2 subunit levels in GluA3\_Ab+ patients ( $*p < 0.05$ , unpaired Student's t-test vs. GluA3\_Ab-, Fig. 1B) and no changes in GluA1 and GluA3 subunits. We considered the occipital cortex of the same patients as an internal control area not affected by this disease. Analysis of postsynaptic fractions (Fig. S1A) and total homogenate (Fig. S1B) obtained from the occipital cortex did not show any significant difference in AMPA receptor subunits between GluA3\_Ab+ or GluA3\_Ab- patients. Overall, these results argue for a biological effect of anti-GluA3 antibodies on the postsynaptic levels of GluA3-containing AMPA receptors.

The mechanisms involved in the synaptic retention of AMPA receptors have been largely elucidated, and protein-protein interaction plays a key role in these events (Diering and Huganir, 2018; Pick and Ziff, 2018). Mainly, 2 scaffolding proteins are involved in receptor insertion/internalization at the postsynaptic membrane, namely PICK1 and GRIP1 (Diering and Huganir, 2018). The first is involved in the regulated removal of AMPA receptors from the synaptic plasma membrane (Hanley, 2008), whereas the second is necessary for the anchorage of the receptor at the postsynaptic density (Anggono and Huganir, 2012). In accordance with a reduced expression of GluA3 at the synapse, PICK1 levels were significantly increased in both fractions (+88% postsynaptic fraction,  $**p = 0.0031$ , unpaired Student's t-test vs. GluA3\_Ab-, Fig. 2A; +47% homogenate,  $*p = 0.0486$  unpaired Student's t-test vs. GluA3\_Ab-, Fig. 2B, respectively). Moreover, we observed in GluA3\_Ab+ patients a decrease of GRIP1 in both the postsynaptic density and in the total

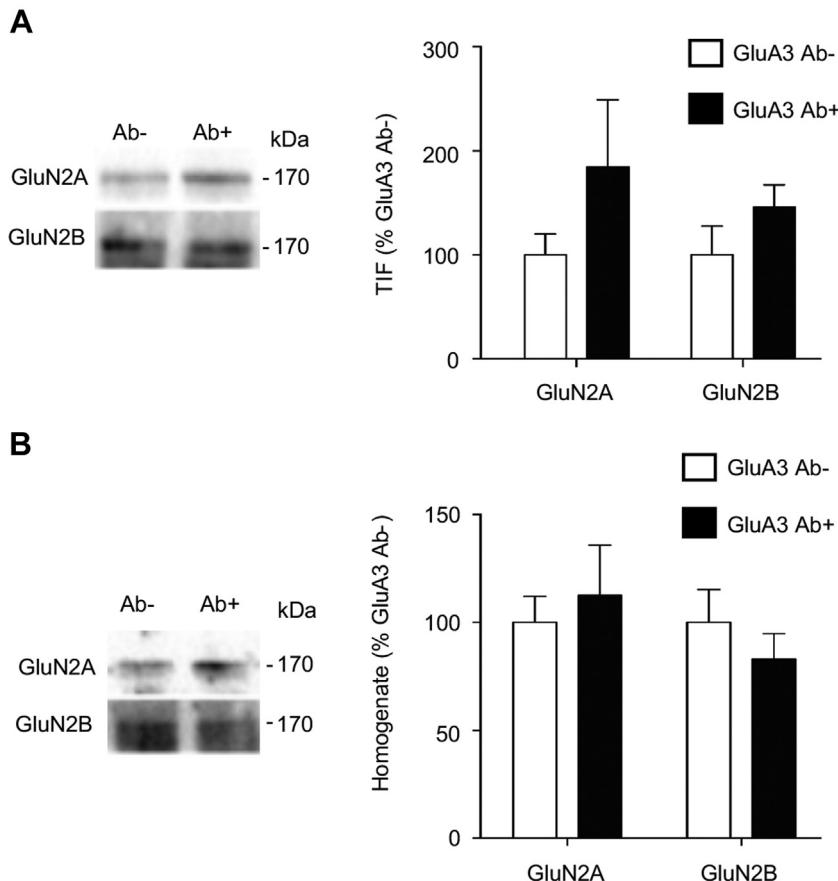
homogenate from temporal cortex (~55% and ~76% respectively), although this effect did not reach a statistical significance (Fig. 2A,  $p = 0.1545$ , unpaired Student's t-test vs. GluA3\_Ab-; Fig. 2B,  $p = 0.1147$ , unpaired Student's t-test vs. GluA3\_Ab-). Accordingly, quantification of PICK1/GRIP1 ratio highlighted a 4-fold increase of GluA3\_Ab+ patients both in the postsynaptic compartment and in the total homogenate ( $*p < 0.05$ , unpaired Student's t-test vs. GluA3\_Ab-, Fig. 2A and B). In agreement with the absence of any alteration of AMPA receptor subunits (Fig. S1A and B), no effect was detected in the levels of both GRIP1 and PICK1 in the occipital cortex of GluA3\_Ab+ (Fig. S2A and B). No significant alteration was observed in GluA3\_Ab+ patients on the synaptic (Fig. 3A) and total levels (Fig. 3B) of N-methyl-D-aspartate (NMDA)-type receptor subunit in the temporal cortex, thus suggesting that GluA3 antibodies target AMPA receptors without interfering with other glutamate receptor subtypes.

### 3.2. Effect of GluA3 antibody on the AMPA-evoked glutamate exocytosis from mice cortical synaptosomes

To further investigate the role of GluA3 autoantibodies on glutamatergic neurotransmission, we evaluated the effects of the CSF from GluA3\_Ab+ FTD patients ( $n = 4$ ) with low [0.100 optical density, 450 nm] to high (0.341 optical density, 450 nm) antibody titer compared with the CSF of GluA3\_Ab- FTD patients ( $n = 4$ ) on the (S)-AMPA-evoked glutamate exocytosis from mice cortical synaptosomes. Glutamate exocytosis was quantified as the release of preloaded [ $^3$ H]D-aspartate ([ $^3$ H]D-Asp), an unmetabolizable analogue of glutamate that allows a reliable measurement of the



**Fig. 2.** Neurobiological effect of GluA3 antibody on AMPA receptor subunit-interacting proteins in the temporal cortex of FTLD-tau patients. Western blot quantification of PICK1, GRIP1, and their ratio in Triton-insoluble postsynaptic fractions (TIF) (A) and total homogenate (B) obtained from the temporal cortex of FTLD-tau patients with (Ab+, closed bars) or without (Ab-, open bars) anti-GluA3 antibodies; left panel: representative blot; right panel: densitometric quantification. Tubulin was used for normalization.  $*p < 0.05$ ,  $**p < 0.01$ , unpaired Student's t-test (mean of 3 experiments with  $n = 3$ ).



**Fig. 3.** Neurobiological effect of GluA3 antibody on NMDA receptor's composition in the temporal cortex of FTLD-tau patients. Western blot quantification of GluN2A and GluN2B subunits in Triton-insoluble postsynaptic fractions (TIF) (A) and total homogenate (B) obtained from the temporal cortex of patients with (Ab+, closed bars) or without (Ab-, open bars) anti-GluA3 antibodies. Left panel: representative blot; right panel: densitometric quantification. Tubulin was used for normalization. (mean of 3 experiments with  $n = 3$ ).

release of glutamate from cortical nerve endings (Grilli et al., 2004). Previous data showed the existence in cortical synaptosomes of presynaptic release-regulating AMPA receptors, the activation of which elicits the  $\text{Ca}^{2+}$ -dependent, exocytotic-like release of glutamate (Pittaluga et al., 1997). Incubation of synaptosomes with the CSF from GluA3\_Ab- patients (1:100 to 1:200 dilutions) failed to significantly modify the AMPA-evoked release of [ $^3\text{H}$ ]D-Asp, whereas incubation of synaptosomes with GluA3\_Ab+ patients CSF reduced the AMPA-evoked tritium release in a dilution-dependent fashion (Fig. 4A). These results were further analyzed by correlating the reduction of the AMPA-induced release efficiency in synaptosomes exposed to selected dilutions (1:200 and 1:100, Fig. 4B and C respectively) of the 8 (4 Ab+ and 4 Ab-) CSF patients and the anti-GluA3 antibody titer. In both cases, the analysis unveiled a strict correlation between the 2 parameters as clearly indicated by the coefficient of linear correlation analysis (1:200,  $r^2 = 0.8764$ ; 1:100,  $r^2 = 0.9072$ ).

Overall, these data indicate that the presence of GluA3 autoantibodies affect glutamatergic neurotransmission at both presynaptic and postsynaptic sites, decreasing glutamate release and altering GluA3-containing AMPA receptor levels.

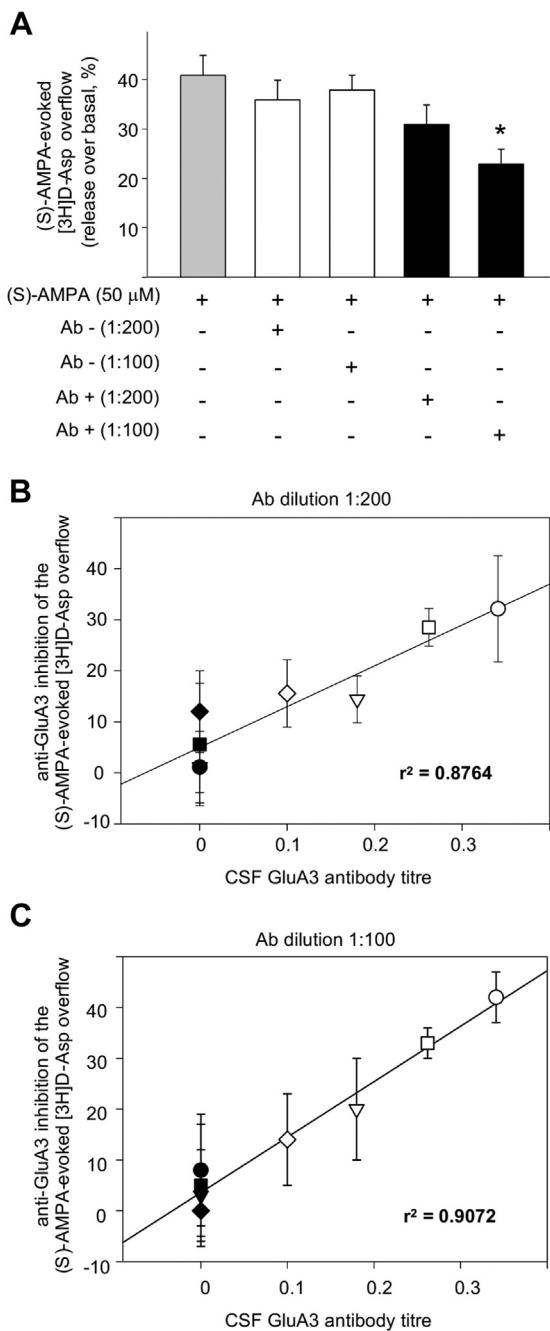
### 3.3. Assessment of glutamatergic intracortical circuits *in vivo* in FTD patients with TMS

It is well known that a physiological AMPA receptor activity plays a pivotal role in the functional regulation of glutamatergic

neurotransmission in the central nervous system (Diering and Huganir, 2018). TMS has become a promising tool to assess specific cortical circuits in the central nervous system (Benussi et al., 2017). Specifically, TMS was used to investigate ICF, a paired-pulse protocol which induces facilitation of the MEP, likely mediated by excitatory glutamate receptors, allowing the indirect and *in vivo* assessment of these circuits (Benussi et al., 2017). Analysis of peak ICF (ISI 7 ms) was significantly reduced in 111 FTD patients (including both GluA3\_Ab+ [ $n = 37$ ] and GluA3\_Ab- [ $n = 74$ ] patients), compared with a group of 70 age-matched healthy controls,  $[F(1,179) = 219.5$ , partial  $\eta^2 = 0.55$ ,  $p < 0.001$ ; Fig. 5A] (see Table 2 for patients' demographic characteristics). Moreover, one-way ANCOVA, corrected for disease severity (FTLD-modified Clinical Dementia Rating Scale), showed a significant difference in ICF between groups  $[F(2,177) = 78.5$ , partial  $\eta^2 = 0.47$ ,  $p < 0.001$ ; Fig. 5B]. Post hoc analysis with Bonferroni corrections showed that ICF was significantly reduced in both GluA3\_Ab+ and GluA3\_Ab- FTD patients compared with healthy controls ( $p < 0.001$ ) and was significantly reduced in GluA3\_Ab+ compared with GluA3\_Ab- patients ( $p = 0.010$ ; Fig. 5B), with a decreased facilitation in GluA3\_Ab+ FTD patients (mean difference of  $0.14 \pm 0.05$ ).

### 3.4. Increased D-/L-serine and L-glutamate content in the CSF and in the serum of FTD patients

To investigate the homeostasis of the glutamatergic neurotransmission in FTD patients, we measured the L-glutamate (L-Glu)



**Fig. 4.** Neurobiological effect of GluA3 antibody on the AMPA-evoked glutamate exocytosis from mice cortical synaptosomes. (A) (S)-AMPA (50 μM)-evoked [<sup>3</sup>H]D-Asp exocytosis from synaptosomes incubated in the absence (gray bar) or in the presence of CSFs (dilution as indicated) from patients without (Ab-, black bar, n = 4) and with Ab+ (white bar, n = 4) anti-GluA3 antibodies. Results are expressed as AMPA-evoked tritium overflow. Data are the means ± SEM of 3 experiments (run in triplicate) for each CSF. \*p < 0.05, one-way ANOVA analysis followed by Dunnett's test. (B and C) Correlation between the Ab- (closed symbols) and Ab+ (open symbols, 1:200 dilution) CSF-induced changes to the AMPA-evoked release of tritium (expressed as % of inhibition) and the respective anti-GluA3 titer for each CSF. The linear regression analysis coefficient ( $r^2$ ) is reported within the plot. (C) as for (B) but the CSF is diluted 1:100. Abbreviation: CSF, cerebrospinal fluid.

content as well as the levels of NMDAR co-agonist D-serine (D-Ser) and its L-enantiomer, L-serine (L-Ser) in CSF of 50 FTD patients and 23 healthy controls (Ctrl) (see Table 2). In addition, we evaluated the systemic metabolism of L-Glu and D-/L-Ser in the serum of the cohort of 40 FTD patients and 13 healthy controls (see Table 2).

HPLC analysis showed a significant increase of D-Ser amount in the CSF of FTD patients compared with control subjects (Mann-Whitney test,  $p = 0.0003$ ; Fig. 6A). Similarly, we observed higher levels of L-serine (L-Ser) in FTD patients, compared with healthy individuals (Mann-Whitney test,  $p = 0.0001$ ; Fig. 6B). Accordingly, no main changes in the D-serine/total serine ratio were found between the 2 groups (Mann-Whitney test,  $p = 0.7104$ ; Fig. 6C). Remarkably, we found a significant increase of L-Glu levels in FTD patients compared with control subjects (Mann-Whitney test,  $p = 0.0004$ ; Fig. 6D).

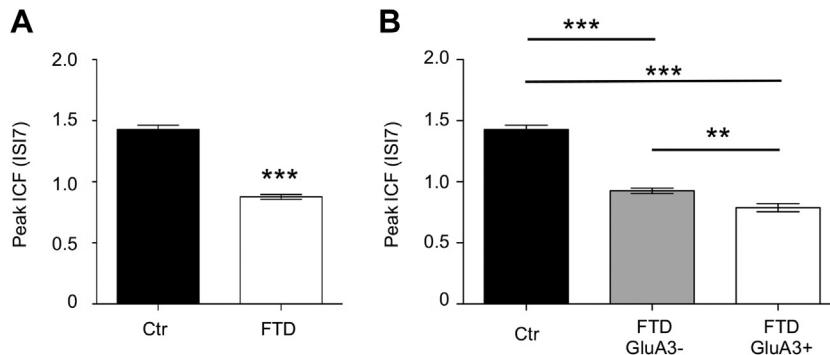
Next, we evaluated the effect induced by the expression of GluA3 autoantibodies on D-/L-Ser and L-Glu levels in the CSF samples of FTD patients. To this aim, we subdivided the FTD group into 2 experimental groups composed by FTD-positive (GluA3\_Ab+, n = 24) and FTD-negative (GluA3\_Ab-, n = 26) subjects for the anti-GluA3 antibody (see Table 2). We found significant increased levels of D-/L-Ser and L-Glu in both GluA3\_Ab+ and GluA3\_Ab- FTD patients, as compared with controls, but we failed to find out significant differences between GluA3\_Ab+ and GluA3\_Ab- subgroups (see Fig. 6E–H; Fig. 6E, Kruskal-Wallis test,  $p = 0.0017$ ; Dunn's test, Ctrl vs. FTD GluA3\_Ab+,  $p = 0.0038$ ; Ctrl vs. FTD GluA3\_Ab-,  $p = 0.0010$ ; Fig. 6F, Kruskal-Wallis test,  $p = 0.0010$ ; Dunn's test, Ctrl vs. FTD GluA3\_Ab+,  $p = 0.0007$ ; Ctrl vs. FTD GluA3\_Ab-,  $p = 0.0021$ ; Fig. 6G, Kruskal-Wallis test,  $p = 0.7792$ ; Fig. 6H, Kruskal-Wallis test,  $p = 0.0024$ ; Dunn's test; Ctrl vs. FTD GluA3\_Ab+,  $p = 0.0029$ ; Ctrl vs. FTD GluA3\_Ab-,  $p = 0.0022$ ).

Differently to what observed in CSF, we found unchanged content of D- and L-Ser in the serum of subjects with FTD compared with control individuals (Fig. 6I, Mann-Whitney test,  $p = 0.8783$ ; Fig. 6J, Mann-Whitney test,  $p = 0.1246$ ). Consequently, the D-serine/total serine ratio was not significantly altered between groups analyzed (Fig. 6K, Mann-Whitney test,  $p = 0.1729$ ). Similarly, Despite an evident trend to increase, statistical analysis revealed no significant alterations of L-Glu levels in the serum of FTD patients compared with control subjects (Fig. 6L, Mann-Whitney test,  $p = 0.0551$ ). Next, we evaluated the effect induced by GluA3 autoantibodies expression on D-/L-Ser and L-Glu levels in the serum of the same cohort of FTD patients (GluA3\_Ab+/GluA3\_Ab-, n = 20 and Ctrl n = 13). HPLC analysis revealed no significant changes in D-/L-Ser content and D-serine/total serine ratio in the serum of both GluA3\_Ab+ and GluA3\_Ab- FTD subjects compared with controls (see Fig. 6M–O; Fig. 6M, Kruskal-Wallis test,  $p = 0.9856$ ; Fig. 6N, Kruskal-Wallis test,  $p = 0.3006$ ; Fig. 6O, Kruskal-Wallis test,  $p = 0.3326$ ). Finally, statistical analysis revealed no significant alterations of L-Glu levels in the serum of FTD patients with or without GluA3 antibody expression, compared with control individuals (Fig. 6P, Kruskal-Wallis test,  $p = 0.1455$ ).

#### 4. Discussion

In the present study, we demonstrated that anti-AMPA antibodies mediate detrimental effects inducing a complex alteration of glutamatergic neurotransmission both in postmortem brain specimens from FTLD-tau patients and *in vivo* in FTD cases (see Fig. 7). In particular, results here presented demonstrate that anti-GluA3 antibodies lead to a reduction in the postsynaptic expression of GluA3-containing AMPA receptors in the temporal cortex of FTLD-tau patients. This evidence is in accordance with our previous *in vitro* studies demonstrating that administration of human anti-GluA3 antibodies results in a significant decrease of the GluA3 subunit levels at postsynaptic sites in both rat primary neurons and in neurons derived from human iPSCs (Borroni et al., 2017).

GluA3 is a highly relevant subunit of AMPA receptors in the brain, and a high proportion of cortical AMPA receptors contain this subunit (Schwenk et al., 2014). Importantly, GluA3-containing



**Fig. 5.** Indirect assessment of glutamatergic intracortical circuits with TMS in FTD patients. (A) Peak ICF (7 ms interstimulus interval) in FTD patients (both GluA3+ and GluA3-) and age-matched healthy controls (Ctr). (B) Peak ICF in FTD GluA3+ and GluA3- patients compared with age-matched healthy controls (Ctr). \*\*\* $p$  < 0.001; \*\* $p$  < 0.005. Abbreviations: FTD, frontotemporal dementia; TMS, transcranial magnetic stimulation.

AMPA receptors are uniformly enriched in the synapse and, only rarely, they are distributed perisynaptically (Jacob and Weinberg, 2015). From a functional point of view, GluA2/GluA3 AMPA receptors are recruited in a constitutive manner to synapses, where they can replace GluA1-containing receptors that are usually added to synaptic membranes during plasticity (Shi et al., 2001). Previous reports obtained in mice models addressed a specific role for GluA3 in Alzheimer's disease. GluA3 knockout mice are protected against A $\beta$ -driven synaptic deficits and memory impairment. In particular, A $\beta$  triggers the synaptic removal of GluA3-containing AMPA receptors (Reinders et al., 2016). Moreover, knocking out of the GRIA3 gene encoding the GluA3 subunit produces alteration of social and aggressive behavior in mice (Adamczyk et al., 2012).

Similar to our data on anti-GluA3 antibodies, a very recent study from Haselmann et al. (2018) showed that human GluA2 antibodies induce AMPA receptor internalization and a consequent decrease of the synaptic abundance GluA2-containing AMPA receptors. This mechanism leads also to an impairment of long-term synaptic plasticity and affects learning and memory. Furthermore, administration of human anti-NMDA antibodies to mice induces a progressive and selective decrease of NMDA receptor synaptic clusters with a similar mechanism (Olivero et al., 2019; Planagumà et al., 2015).

It is well known that the synaptic pool of AMPA receptors is highly dynamic, undergoing activity-dependent endocytosis/exocytosis events through PDZ-mediated interaction with GRIP1 and PICK1 (Anggono and Huganir, 2012; Diering and Huganir,

2018). PICK1 mediates the depletion of GluA2/3 AMPA receptors from synapses (Kim et al., 2001; Terashima et al., 2008), whereas GRIP1 anchors the receptors in the postsynaptic density (Anggono and Huganir, 2012). Here, we show that the reduced GluA3 localization at synapses in the temporal cortex of anti-GluA3-positive FTLD-tau patients is accompanied by a 4-fold increased PICK1/GRIP1 ratio. This observation indicates that GluA3 antibodies promote endocytosis of the receptor subunit, probably through interaction with PICK1. Moreover, we observed that the presence of GluA3\_Ab+ decreases glutamate release from synaptosomes in a dose-dependent manner. Accordingly, it is possible to state that the presence of GluA3 antibodies can affect glutamatergic neurotransmission acting both at the presynaptic terminal, by reducing glutamate release, and at dendritic spines lowering the availability in the postsynaptic membranes of AMPA-type glutamate receptors.

The aforementioned results were further corroborated by neurophysiological techniques in FTD patients, confirming the harmful effect of anti-GluA3 antibodies. Taking into account the key role of AMPA receptors in the regulation of glutamatergic neurotransmission (Diering and Huganir, 2018), in the present study we used TMS to investigate ICF and to perform an in vivo assessment of excitatory glutamatergic circuits (Benussi et al., 2017). ICF has been previously shown to be deficient both in sporadic and genetic FTD patients compared with healthy controls (Benussi et al., 2017) and to correlate with disease progression (Benussi et al., 2019). Here, we observed a significant difference in ICF not only between healthy

**Table 2**

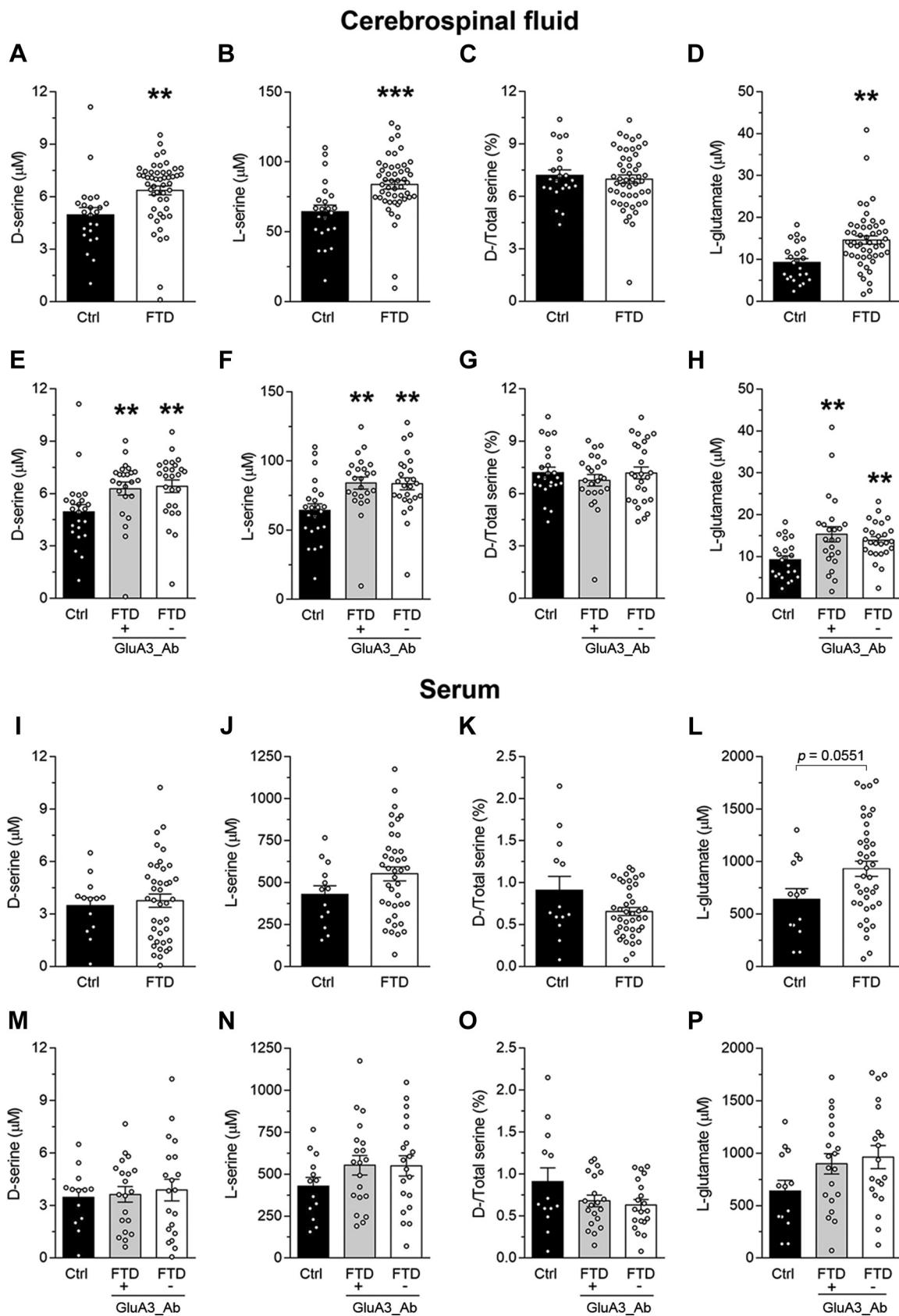
Demographic characteristics of FTD patients and control subjects that underwent to TMS analysis, CSF, and serum dosages

Variable	Controls		FTD patients		
	Total		Total	GluA3_Ab+	GluA3_Ab-
<b>TMS parameters evaluation</b>					
Subjects (N)	70		111	37	74
Age (mean $\pm$ SEM of years)	68.0 $\pm$ 1.1		65.2 $\pm$ 0.8	65.0 $\pm$ 1.6	65.3 $\pm$ 0.9
Gender	27 M, 43 F		61 M, 50 F	20 M, 17 F	41 M, 33 F
<b>CSF dosages</b>					
Subjects (N)	23		50	24	26
Age (mean $\pm$ SEM of years)	72.3 $\pm$ 2.8		68.0 $\pm$ 1.1 <sup>a</sup>	67.5 $\pm$ 1.9 <sup>a</sup>	68.5 $\pm$ 1.3 <sup>a</sup>
Gender	17 M, 6 F		30 M, 20 F	16 M, 8 F	14 M, 12 F
<b>Serum dosages</b>					
Subjects (N)	13		40	20	20
Age (mean $\pm$ SEM of years)	69.4 $\pm$ 2.1		67.5 $\pm$ 1.3	67.4 $\pm$ 2.2	67.5 $\pm$ 1.6
Gender	7 M, 6 F		24 M, 16 F	13 M, 7 F	11 M, 9 F

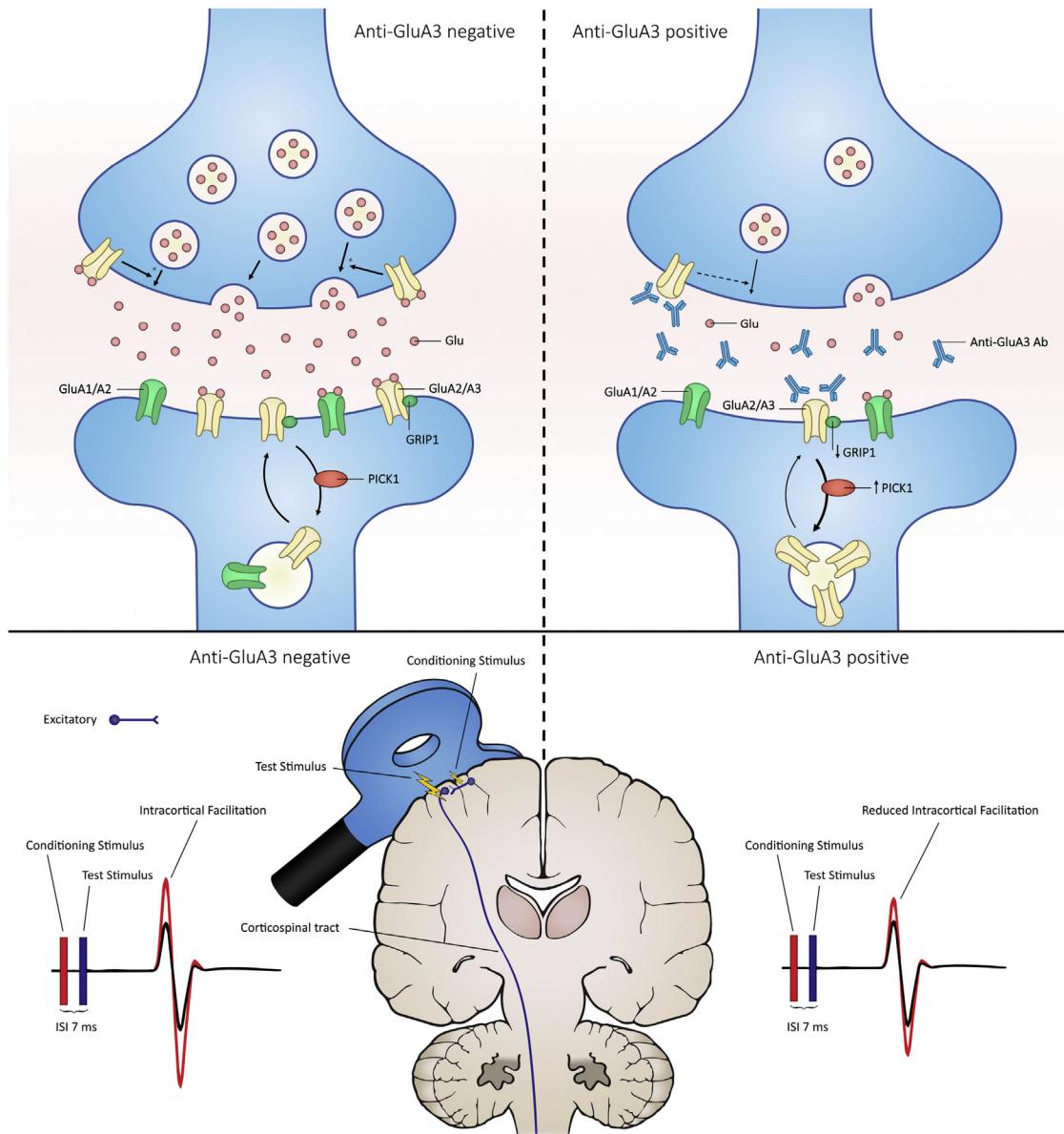
Age effect was evaluated by the Mann Whitney test or Kruskal-Wallis test, followed by Dunn's test.

Key: N, number; M, males; F, females; U, users; NU, nonusers; N/A, not available information; FTLD, frontotemporal dementia; CSF, cerebrospinal fluid; TMS, transcranial magnetic stimulation.

<sup>a</sup>  $p$  < 0.01, compared with controls. Gender was evaluated by the chi-square test.



**Fig. 6.** Analysis of D-/L-serine and L-glutamate content in the CSF and serum of FTD patients. (A–D) Content of (A) D-serine, (B) L-serine, (C) D-serine/total serine ratio, and (D) L-glutamate in the CSF of the entire cohort of FTD patients (FTD,  $n = 50$ ) and control subjects (Ctrl,  $n = 23$ ). \*\* $p < 0.01$ , \*\*\* $p < 0.0001$ , compared with the control group (Mann-Whitney test). (E–H) Amount of (E) D-serine, (F) L-serine, (G) D-serine/total serine ratio, and (H) L-glutamate in the CSF of anti-GluA3-positive (+) and anti-GluA3-negative (-) FTD patients (FTD GluA3\_Ab+,  $n = 24$ ; FTD GluA3\_Ab-,  $n = 26$ ) and control individuals (Ctrl,  $n = 23$ ). \*\* $p < 0.01$ , compared with the control group (Dunn's test). (I–L) Levels of (I) D-



**Fig. 7.** Schematic representation of the molecular and functional effects induced by the presence of anti-GluA3 antibodies. (Upper panels) Effect of GluA3 antibody on synaptic AMPA receptor subunit composition and glutamate release at excitatory glutamatergic synapses. (Lower panels) Reduced ICF as measured by TMS in FTD patients with anti-GluA3 antibodies as compared to FTD patients without anti-GluA3 antibodies. Abbreviations: FTD, frontotemporal dementia; ICF, intracortical facilitation; TMS, transcranial magnetic stimulation.

controls and FTD patients but also within FTD patients between GluA3<sub>Ab+</sub> and GluA3<sub>Ab-</sub> patients. This observation may be predicting of a more pronounced impairment of glutamatergic neurotransmission in presence of the GluA3 antibody.

Finally, we carefully characterized L-Glu, L-Ser, and D-Ser levels in both CSF and serum of FTD patients. A recent report from Madeira et al. (2018), performed on a limited number ( $N = 14$ ) of FTD patients, found a mild increase in D- and L-serine levels in Alzheimer's disease patients but not in FTD-affected subjects. Conversely, in the present work, we detect that the altered synaptic AMPA receptor composition and the impaired glutamatergic

neurotransmission observed in FTD patients were accompanied by a significant increase in the CSF levels of D-Ser, L-Ser, and L-Glu. Differently to what observed in CSF, although an evident trend to increase we found unchanged levels of L-Glu and D- and L-Ser in the serum of subjects with FTD compared with control individuals. Notably, the presence of anti-GluA3 antibodies does not induce any significant difference in the levels of L-Glu, D-Ser, and L-Ser. These results may indicate a compensatory process aimed to balance the reduced AMPA-mediated transmission in FTD patients. However, even if these neurochemical results combined with the TMS analysis can represent a novel potential biomarker in FTD, further

serine, (J) L-serine, (K) D-serine/total serine ratio, and (L) L-glutamate in the serum of the entire cohort of FTD patients (FTD,  $n = 40$ ) and control subjects (Ctrl,  $n = 13$ ). (M–P) Concentration of (M) D-serine, (N) L-serine, (O) D-serine/total serine ratio, and (P) L-glutamate in the serum of anti-GluA3-positive (+) and anti-GluA3-negative (-) FTD patients (FTD\_GluA3<sub>Ab+</sub>,  $n = 20$ ; FTD\_GluA3<sub>Ab-</sub>,  $n = 20$ ) and control individuals (Ctrl,  $n = 13$ ). In each sample, all free amino acids were detected in a single run by HPLC and expressed as  $\mu\text{M}$ , whereas the ratio is expressed as percentage (%). Dots represent the single subjects' values, whereas bars illustrate the means  $\pm$  SEM. Abbreviations: CSF, cerebrospinal fluid; FTD, frontotemporal dementia.

studies are surely needed to evaluate the mechanisms involved in these events.

Overall, our results are in agreement with several recent pre-clinical (Decker et al., 2016; Longhena et al., 2017; Udagawa et al., 2015) and clinical studies (Leuzy et al., 2016; Benussi et al., 2017, 2019) indicating a key role of glutamate receptors and glutamatergic neurotransmission in the pathogenesis of FTD and assign a specific role for anti-GluA3 antibodies in a subgroup of these patients. As in the other autoimmune disorders of the central nervous system, as Rasmussen or anti-NMDA encephalitis (Esposito et al., 2019), here we observe a selective neuronal vulnerability confined to the temporal cortex, while the occipital cortex is spared. The mechanism(s) leading to autoimmune response to AMPA receptors in FTD needs further investigations. It might be hypothesized an immune-mediated genetic enrichment, particularly within the human leukocyte antigen region, or a predisposition related to specific protein misfolding. However, we cannot conclude whether anti-GluA3 antibodies are a consequence of the ongoing pathological process or, instead, a cause. Further studies are required to clarify this issue. Despite this, restoration of a physiological glutamatergic transmission should be taken into account and might be obtained by acting at the AMPA-type glutamate receptors as well as at the immune system.

We acknowledge that this study entails some limitations. Above all, we considered only FTLD-tau cases for those experiments conducted on autopsy specimens to have a more homogeneous sample. It would be of great interest to evaluate the presence and eventually the effect of anti-AMPA antibodies in FTLD-TDP43 cases. This would allow us to compare the results herein obtained with those of other proteinopathies related to FTLD and potentially to distinguish tau and TDP-43 cases if results differ in autoantibody expression. Moreover, a careful analysis of the rates of clinical progression and glutamatergic neurotransmission impairment over time in FTD patients with or without anti-GluA3 antibodies is required. Finally, the association between the presence of anti-GluA3 antibodies and autoimmune disease during lifetime needs to be further investigated.

In conclusion, even if further studies are needed to evaluate the molecular mechanisms involved in these events, we can hypothesize that an immune system dysregulation might result into an abnormal production of autoantibodies directed against the GluA3 subunit, causing a complex dysfunction in glutamatergic transmission. Accordingly, the role of glutamate in the brain circuits represents an interesting and innovative approach to (i) better understand the neurodegenerative process in FTD and (ii) discover new strategies to revert or slow disease progression through the modulation of glutamatergic pathway via immune system.

## Disclosure

The authors declare that they have no competing interests.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.neurobiolaging.2019.10.015>.

## References

- Adamczyk, A., Mejias, R., Takamiya, K., Yocom, J., Krasnova, I.N., Calderon, J., Cadet, J.L., Huganir, R.L., Pletnikov, M.V., Wang, T., 2012. GluA3-deficiency in mice is associated with increased social and aggressive behavior and elevated dopamine in striatum. *Behav. Brain Res.* 229, 265–272.
- Alberici, A., Cristillo, V., Gazzina, S., Benussi, A., Padovani, A., Borroni, B., 2018. Autoimmunity and frontotemporal dementia. *Curr. Alzheimer Res.* 15, 602–609.
- Anggono, V., Huganir, R.L., 2012. Regulation of AMPA receptor trafficking and synaptic plasticity. *Curr. Opin. Neurobiol.* 22, 461–469.
- Baker, M., Mackenzie, I.R., Pickering-Brown, S.M., Gass, J., Rademakers, R., Lindholm, C., Snowden, J., Adamson, J., Sadovnick, A.D., Rollinson, S., Cannon, A., Dwosh, E., Neary, D., Melquist, S., Richardson, A., Dickson, D., Berger, Z., Eriksen, J., Robinson, T., Zehr, C., Dickey, C.A., Crook, R., McGowan, E., Mann, D., Boeve, B., Feldman, H., Hutton, M., 2006. Mutations in progranulin cause tau-negative frontotemporal dementia linked to chromosome 17. *Nature* 442, 916–919.
- Benussi, A., Padovani, A., Borroni, B., 2015. Phenotypic heterogeneity of monogenic frontotemporal dementia. *Front Aging Neurosci.* 7, 171.
- Benussi, A., Cosseddu, M., Filaretto, I., Dell'Era, V., Archetti, S., Sofia Cotelli, M., Micheli, A., Padovani, A., Borroni, B., 2016. Impaired long-term potentiation-like cortical plasticity in presymptomatic genetic frontotemporal dementia. *Ann. Neurol.* 80, 472–476.
- Benussi, A., Di Lorenzo, F., Dell'Era, V., Cosseddu, M., Alberici, A., Caratozzolo, S., Cotelli, M.S., Micheli, A., Rozzini, L., Depari, A., Flaminini, A., Ponzo, V., Martorana, A., Caltagirone, C., Padovani, A., Koch, G., Borroni, B., 2017. Transcranial magnetic stimulation distinguishes Alzheimer disease from frontotemporal dementia. *Neurology* 89, 665–672.
- Benussi, A., Gazzina, S., Premi, E., Cosseddu, M., Archetti, S., Dell'Era, V., Cantoni, V., Cotelli, M.S., Alberici, A., Micheli, A., Benussi, L., Ghidoni, R., Padovani, A., Borroni, B., 2019. Clinical and biomarker changes in presymptomatic genetic frontotemporal dementia. *Neurobiol. Aging* 76, 133–140.
- Borroni, B., Stanic, J., Verpelli, C., Mellone, M., Bonomi, E., Alberici, A., Bernasconi, P., Culotta, L., Zianni, E., Archetti, S., Manes, M., Gazzina, S., Ghidoni, R., Benussi, L., Stuani, C., Di Luca, M., Sala, C., Buratti, E., Padovani, A., Gardoni, F., 2017. Anti-AMPA GluA3 antibodies in Frontotemporal dementia: a new molecular target. *Sci. Rep.* 7, 6723.
- Broe, I., Karch, C.M., Wen, N., Fan, C.C., Wang, Y., Tan, C.H., Kouri, N., Ross, O.A., Höglunger, G.U., Muller, U., Hardy, J., International FTD-Genomics Consortium, Momeni, P., Hess, C.P., Dillon, W.P., Miller, Z.A., Bonham, L.W., Rabinovici, G.D., Rosen, H.J., Schellenberg, G.D., Franke, A., Karlsen, T.H., Veldink, J.H., Ferrari, R., Yokoyama, J.S., Miller, B.L., Andreassen, O.A., Dale, A.M., Desikan, R.S., Sugrue, L.P., 2018. Immune-related genetic enrichment in frontotemporal dementia: an analysis of genome-wide association studies. *PLoS Med.* 15, e1002487.
- Cavazzana, I., Alberici, A., Bonomi, E., Ottaviani, R., Kumar, R., Archetti, S., Manes, M., Cosseddu, M., Buratti, E., Padovani, A., Tincani, A., Franceschini, F., Borroni, B., 2018. Antinuclear antibodies in Frontotemporal Dementia: the tip's of autoimmunity iceberg? *J. Neuroimmunol.* 325, 61–63.
- Cruts, M., Gijselink, I., van der Zee, J., Engelborghs, S., Wils, H., Pirici, D., Rademakers, R., Vandenberghe, R., Dermaut, B., Martin, J.J., van Duijn, C., Peeters, K., Sciot, R., Santens, P., De Pooter, T., Mattheijssens, M., Van den Broeck, M., Cuijt, I., Vennekens, K., De Deyn, P.P., Kumar-Singh, S., Van Broeckhoven, C., 2006. Null mutations in progranulin cause ubiquitin-positive frontotemporal dementia linked to chromosome 17q21. *Nature* 442, 920–924.
- Decker, J.M., Krüger, L., Sydow, A., Dennissen, F.J., Siskova, Z., Mandelkow, E., 2016. The Tau/A152T mutation, a risk factor for frontotemporal-spectrum disorders, leads to NR2B receptor-mediated excitotoxicity. *EMBO Rep.* 17, 552–569.
- DeJesus-Hernandez, M., Mackenzie, I.R., Boeve, B.F., Boxer, A.L., Baker, M., Rutherford, N.J., Nicholson, A.M., Finch, N.A., Flynn, H., Adamson, J., Kouri, N., Wojtas, A., Sengdy, P., Hsiung, G.Y., Karydas, A., Seeley, W.W., Josephs, K.A., Coppola, G., Geschwind, D.H., Wszolek, Z.K., Feldman, H., Knopman, D.S., Petersen, R.C., Miller, B.L., Dickson, D.W., Boylan, K.B., Graff-Radford, N.R., Rademakers, R., 2011. Expanded GGGGCC hexanucleotide repeat in noncoding region of C9ORF72 causes chromosome 9p-linked FTD and ALS. *Neuron* 72, 245–256.
- Diering, G.H., Huganir, R.L., 2018. The AMPA receptor code of synaptic plasticity. *Neuron* 100, 314–329.
- Esposito, S., Principi, N., Calabresi, P., Rigante, D., 2019. An evolving redefinition of autoimmune encephalitis. *Autoimmun. Rev.* 18, 155–163.
- Ferrari, R., Hernandez, D.G., Nalls, M.A., Rohrer, J.D., Ramasamy, A., Kwok, J.B., Dobson-Stone, C., Brooks, W.S., Schofield, P.R., Halliday, G.M., Hodges, J.R., Piguet, O., Bartley, L., Thompson, E., Haan, E., Hernández, I., Ruiz, A., Boada, M., Borroni, B., Padovani, A., Cruchaga, C., Cairns, N.J., Benussi, L., Binetti, G., Ghidoni, R., Forloni, G., Galimberti, D., Fenoglio, C., Serpente, M., Scarpi, E., Clarimón, J., Lleó, A., Blesa, R., Waldö, M.L., Nilsson, K., Nilsson, C., Mackenzie, I.R., Hsiung, G.Y., Mann, D.M., Grafman, J., Morris, C.M., Attems, J., Griffiths, T.D., McKeith, I.G., Thomas, A.J., Pietrini, P., Huey, E.D., Wassermann, E.M., Babior, A., Jaros, E., Tierney, M.C., Pastor, P., Razquin, C., Ortega-Cubero, S., Alonso, E., Perneczky, R., Diehl-Schmid, J., Alexopoulos, P., Kurz, A., Rainero, I., Rubino, E., Pinessi, L., Rogaeva, E., St George-Hyslop, P., Rossi, G., Tagliavini, F., Giaccone, G., Rowe, J.B., Schlachetzki, J.C., Uphill, J., Collinge, J., Mead, S., Danek, A., Van Deerlin, V.M., Grossman, M., Trojanowski, J.Q., van der Zee, J., Deschamps, W., Van Langenhove, T., Cruts, M.,

- Van Broeckhoven, C., Cappa, S.F., Le Ber, I., Hannequin, D., Golfier, V., Vercelletto, M., Brice, A., Nacmias, B., Sorbi, S., Bagnoli, S., Piaceri, I., Nielsen, J.E., Hjermind, L.E., Riemenschneider, M., Mayhaus, M., Ibach, B., Gasparoni, G., Pichler, S., Gu, W., Rossor, M.N., Fox, N.C., Warren, J.D., Spillantini, M.G., Morris, H.R., Rizzu, P., Heutink, P., Snowden, J.S., Rollinson, S., Richardson, A., Gerhard, A., Bruni, A.C., Maletta, R., Frangipane, F., Cupidi, C., Bernardi, L., Afossi, M., Gallo, M., Conidi, M.E., Smirne, N., Rademakers, R., Baker, M., Dickson, D.W., Graff-Radford, N.R., Petersen, R.C., Knopman, D., Josephs, K.A., Boeve, B.F., Parisi, J.E., Seeley, W.W., Miller, B.L., Karydas, A.M., Rosen, H., van Swieten, J.C., Dopper, E.G., Seelaar, H., Pijnenburg, Y.A., Scheltens, P., Logroscino, G., Capozzo, R., Novelli, V., Puca, A.A., Franceschi, M., Postiglione, A., Milan, G., Sorrentino, P., Kristiansen, M., Chiang, H.H., Graff, C., Pasquier, F., Rollin, A., Deramecourt, V., Lebert, F., Kapogiannis, D., Ferrucci, L., Pickering-Brown, S., Singleton, A.B., Hardy, J., Momeni, P., 2014. Frontotemporal dementia and its subtypes: a genome-wide association study. *Lancet Neurol.* 13, 686–699.
- Gorno-Tempini, M.L., Hillis, A.E., Weintraub, S., Kertesz, A., Mendez, M., Cappa, S.F., Ogar, J.M., Rohrer, J.D., Black, S., Boeve, B.F., Manes, F., Dronkers, N.F., Vandenberghe, R., Rascovsky, K., Patterson, K., Miller, B.L., Knopman, D.S., Hodges, J.R., Mesulam, M.M., Grossman, M., 2011. Classification of primary progressive aphasia and its variants. *Neurology* 76, 1006–1014.
- Grilli, M., Raiteri, L., Pittaluga, A., 2004. Somatostatin inhibits glutamate release from mouse cerebrocortical nerve endings through presynaptic sst<sub>2</sub> receptor linked to the adenylyl cyclase-protein kinase A pathway. *Neuropharmacology* 46, 388–396.
- Hanley, J.G., 2008. PICK1: a multi-talented modulator of AMPA receptor trafficking. *Pharmacol. Ther.* 118, 152–160.
- Haselmann, H., Mannara, F., Werner, C., Planagumà, J., Miguez-Cabello, F., Schmidl, L., Grünewald, B., Petit-Pedrol, M., Kirmse, K., Classen, J., Demir, F., Klöcker, N., Soto, D., Doose, S., Dalmau, J., Hallermann, S., Geis, C., 2018. Human autoantibodies against the AMPA receptor subunit GluA2 induce receptor reorganization and memory dysfunction. *Neuron* 100, 91–105.
- Hodges, J.R., Piguet, O., 2018. Progress and challenges in frontotemporal dementia research: a 20-year review. *J. Alzheimers Dis.* 62, 1467–1480.
- Hutton, M., Lendon, C.L., Rizzu, P., Baker, M., Froelich, S., Houlden, H., Pickering-Brown, S., Chakraverty, S., Isaacs, A., Grover, A., Hackett, J., Adamson, J., Lincoln, S., Dickson, D., Davies, P., Petersen, R.C., Stevens, M., de Graaff, E., Wauters, E., van Baren, J., Hillebrand, M., Jootse, M., Kwon, J.M., Nowotny, P., Che, L.K., Norton, J., Morris, J.C., Reed, L.A., Trojanowski, J., Basun, H., Lannfelt, L., Neystat, M., Fahn, S., Dark, F., Tannenberg, T., Dodd, P.R., Hayward, N., Kwok, J.B., Schofield, P.R., Andreadis, A., Snowden, J., Craufurd, D., Neary, D., Owen, F., Oostra, B.A., Hardy, J., Goate, A., van Swieten, J., Mann, D., Lynch, T., Heutink, P., 1998. Association of missense and 5'-splice-site mutations in tau with the inherited dementia FTDP-17. *Nature* 393, 702–705.
- Jacob, A.I., Weinberg, R.J., 2015. The organization of AMPA receptor subunits at the postsynaptic membrane. *Hippocampus* 25, 798–812.
- Kim, C.H., Chung, H.J., Lee, H.K., Huganir, R.L., 2001. Interaction of the AMPA receptor subunit GluR2/3 with PDZ domains regulates hippocampal long-term depression. *Proc. Natl. Acad. Sci. U. S. A.* 98, 11725–11730.
- Leuzy, A., Zimmer, E.R., Dubois, J., Pruessner, J., Cooperman, C., Soucy, J.P., Kostikov, A., Schirmacher, E., Désautels, R., Gauthier, S., Rosa-Neto, P., 2016. In vivo characterization of metabotropic glutamate receptor type 5 abnormalities in behavioral variant FTD. *Brain Struct. Funct.* 221, 1387–1402.
- Longhena, F., Zaltieri, M., Grigoletto, J., Faustini, G., La Via, L., Ghidoni, R., Benussi, L., Missale, C., Spano, P., Bellucci, A., 2017. Depletion of progranulin reduces GluN2B-containing NMDA receptor density, tau phosphorylation, and dendritic Arborization in mouse primary cortical neurons. *J. Pharmacol. Exp. Ther.* 363, 164–175.
- Mackenzie, I.R., Neumann, M., Bigio, E.H., Cairns, N.J., Alfuzoor, I., Kril, J., Kovacs, G.G., Ghetti, B., Halliday, G., Holm, I.E., Ince, P.G., Kamphorst, W., Revesz, T., Rozenmuller, A.J., Kumar-Singh, S., Akiyama, H., Baborie, A., Spina, S., Dickson, D.W., Trojanowski, J.Q., Mann, D.M., 2010. Nomenclature and nosology for neuropathologic subtypes of frontotemporal lobar degeneration: an update. *Acta Neuropathol.* 119, 1–4.
- Mackenzie, I.R., Neumann, M., Baborie, A., Sampathu, D.M., Du Plessis, D., Jaros, E., Perry, R.H., Trojanowski, J.Q., Mann, D.M., Lee, V.M., 2011. A harmonized classification system for FTLD-TDP pathology. *Acta Neuropathol.* 122, 111–113.
- Madeira, C., Vargas-Lopes, C., Brandão, C.O., Reis, T., Laks, J., Panizzutti, R., Ferreira, S.T., 2018. Elevated glutamate and glutamine levels in the cerebrospinal fluid of patients with probable Alzheimer's disease and depression. *Front Psychiatry* 9, 561.
- Mantegazza, R., Bernasconi, P., Baggi, F., Spreafico, R., Ragona, F., Antozzi, C., Bernardi, G., Granata, T., 2002. Antibodies against GluR3 peptides are not specific for Rasmussen's encephalitis but are also present in epilepsy patients with severe, early onset disease and intractable seizures. *J. Neuroimmunol.* 131, 179–185.
- Marcello, E., Saraceno, C., Musardo, S., Vara, H., de la Fuente, A.G., Pelucchi, S., Di Marino, D., Borroni, B., Tramontano, A., Pérez-Otaño, I., Padovani, A., Giustetto, M., Gardoni, F., Di Luca, M., 2013. Endocytosis of synaptic ADAM10 in neuronal plasticity and Alzheimer's disease. *J. Clin. Invest.* 123, 2523–2538.
- Miller, Z.A., Rankin, K.P., Graff-Radford, N.R., Takada, L.T., Sturm, V.E., Cleveland, C.M., Criswell, L.A., Jaeger, P.A., Stan, T., Heggeli, K.A., Hsu, S.C., Karydas, A., Khan, B.K., Grinberg, L.T., Gorno-Tempini, M.L., Boxer, A.L., Rosen, H.J., Kramer, J.H., Coppola, G., Geschwind, D.H., Rademakers, R., Seeley, W.W., Graff-Radford, N.R., Miller, B.L., 2016. Increased prevalence of autoimmune disease within C9 and FTD/MND cohorts: completing the picture. *Neurol. Neuroimmunol Neuroinflamm* 3, e301.
- Murley, A.G., Rowe, J.B., 2018. Neurotransmitter deficits from frontotemporal lobar degeneration. *Brain* 141, 1263–1285.
- Nuzzo, T., Punzo, D., Devoto, P., Rosini, E., Paciotti, S., Sacchi, S., Li, Q., Thiolat, M.L., Vega, C., Carella, M., Carta, M., Gardoni, F., Calabresi, P., Pollegioni, L., Bezard, E., Parnetti, L., Errico, F., Usiello, A., 2019. The levels of the NMDA receptor co-agonist D-serine are reduced in the substantia nigra of MPTP-lesioned macaques and in the cerebrospinal fluid of Parkinson's disease patients. *Sci. Rep.* 9, 8898.
- Oh, M.C., Derkach, V.A., Guire, E.S., Soderling, T.R., 2006. Extrasynaptic membrane trafficking regulated by GluR1 serine 845 phosphorylation primes AMPA receptors for long-term potentiation. *J. Biol. Chem.* 281, 752–758.
- Olivero, G., Vergassola, M., Cisani, F., Usai, C., Pittaluga, A., 2019. Immuno-pharmacological characterization of presynaptic GluN3A-containing NMDA autoreceptors: relevance to anti-NMDA receptor autoimmune diseases. *Mol. Neurobiol.* 56, 6142–6155.
- Pick, J.E., Ziff, E.B., 2018. Regulation of AMPA receptor trafficking and exit from the endoplasmic reticulum. *Mol. Cell Neurosci* 91, 3–9.
- Pittaluga, A., 2016. Presynaptic release-regulating mGlu1 receptors in central nervous system. *Front Pharmacol.* 7, 295.
- Pittaluga, A., Bonfanti, A., Raiteri, M., 1997. Differential desensitization of ionotropic non-NMDA receptors having distinct neuronal location and function. *Naunyn Schmiedebergs Arch. Pharmacol.* 356, 29–38.
- Planagumà, J., Leyboldt, F., Mannara, F., Gutiérrez-Cuesta, J., Martín-García, E., Aguilar, E., Titulaer, M.J., Petit-Pedrol, M., Jain, A., Balice-Gordon, R., Lakadamyali, M., Graus, F., Maldonado, R., Dalmau, J., 2015. Human N-methyl D-aspartate receptor antibodies alter memory and behaviour in mice. *Brain* 138, 94–109.
- Rascovsky, K., Hodges, J.R., Knopman, D., Mendez, M.F., Kramer, J.H., Neuhaus, J., van Swieten, J.C., Seelaar, H., Dopper, E.G., Onyike, C.U., Hillis, A.E., Josephs, K.A., Boeve, B.F., Kertesz, A., Seeley, W.W., Rankin, K.P., Johnson, J.K., Gorno-Tempini, M.L., Rosen, H., Prioleau-Latham, C.E., Lee, A., Kipps, C.M., Lillo, P., Piguet, O., Rohrer, J.D., Rossor, M.N., Warren, J.D., Fox, N.C., Galasko, D., Salmon, D.P., Black, S.E., Mesulam, M., Weintraub, S., Dickerson, B.C., Diehl-Schmid, J., Pasquier, F., Deramecourt, V., Lebert, F., Pijnenburg, Y., Chow, T.W., Manes, F., Grafman, J., Cappa, S.F., Freedman, M., Grossman, M., Miller, B.L., 2011. Sensitivity of revised diagnostic criteria for the behavioural variant of frontotemporal dementia. *Brain* 134, 2456–2477.
- Reinders, N.R., Pao, Y., Renner, M.C., da Silva-Matos, C.M., Lodder, T.R., Malinow, R., Kessels, H.W., 2016. Amyloid-β effects on synapses and memory require AMPA receptor subunit GluA3. *Proc. Natl. Acad. Sci. U. S. A.* 113, E6526–E6534.
- Renton, A.E., Majounie, E., Waite, A., Simón-Sánchez, J., Rollinson, S., Gibbs, J.R., Schymick, J.C., Laaksovirta, H., van Swieten, J.C., Myllykangas, L., Kalimo, H., Paetau, A., Abramzon, Y., Remes, A.M., Kaganovich, A., Scholz, S.W., Duckworth, J., Ding, J., Harmer, D.W., Hernandez, D.G., Johnson, J.O., Mok, K., Ryten, M., Trabzuni, D., Guerreiro, R.J., Orrell, R.W., Neal, J., Murray, A., Pearson, J., Jansen, I.E., Sondervan, D., Seelaar, H., Blake, D., Young, K., Halliwell, N., Callister, J.B., Toulson, G., Richardson, A., Gerhard, A., Snowden, J., Mann, D., Neary, D., Nalls, M.A., Peirlinckx, T., Jansson, L., Isoviita, V.M., Kaivorinne, A.L., Hölttä-Vuori, M., Ikonen, E., Sulikava, R., Benatar, M., Wuu, J., Chiò, A., Restagno, G., Borghero, G., Sabatelli, M., ITALSGEN Consortium, Heckerman, D., Rogeava, E., Zinman, L., Rothstein, J.D., Sendtner, M., Drepper, C., Eichler, E.E., Alkan, C., Abdullaev, Z., Pack, S.D., Dutra, A., Pak, E., Hardy, J., Singleton, A., Williams, N.M., Heutink, P., Pickering-Brown, S., Morris, H.R., Tienari, P.J., Traynor, B.J., 2011. A hexanucleotide repeat expansion in C9ORF72 is the cause of chromosome 9p21-linked ALS-FTD. *Neuron* 72, 257–268.
- Rohrer, J.D., Guerreiro, R., Vandervoort, J., Uphill, J., Reiman, D., Beck, J., Isaacs, A.M., Authier, A., Ferrari, R., Fox, N.C., Mackenzie, I.R., Warren, J.D., de Silva, R., Holton, J., Revesz, T., Hardy, J., Mead, S., Rossor, M.N., 2009. The heritability and genetics of frontotemporal lobar degeneration. *Neurology* 73, 1451–1456.
- Rohrer, J.D., Nicholas, J.M., Cash, D.M., van Swieten, J., Dopper, E., Jiskoot, L., van Minkelen, R., Rombouts, S.A., Cardoso, M.J., Clegg, S., Espak, M., Mead, S., Thomas, D.L., De Vita, E., Masellis, M., Black, S.E., Freedman, M., Keren, R., MacIntosh, B.J., Rogeava, E., Tang-Wai, D., Tartaglia, M.C., Laforce Jr., R., Tagliavini, F., Tiraboschi, P., Redaelli, V., Prioni, S., Grisoli, M., Borroni, B., Padovani, A., Galimberti, D., Scarpini, E., Arighi, A., Fumagalli, G., Rowe, J.B., Coyle-Gilchrist, I., Graff, C., Fallström, M., Jelic, V., Ståhlbom, A.K., Andersson, C., Thonberg, H., Lilius, L., Frisoni, G.B., Pievani, M., Bocchetta, M., Benussi, L., Ghidoni, R., Finger, E., Sorbi, S., Nacmias, B., Lombardi, G., Polito, C., Warren, J.D., Ourselin, S., Fox, N.C., Rossor, M.N., Binetti, G., 2015. Presymptomatic cognitive and neuroanatomical changes in genetic frontotemporal dementia in the Genetic Frontotemporal dementia Initiative (GENFI) study: a cross-sectional analysis. *Lancet Neurol.* 14, 253–262.
- Schwenk, J., Baehrens, D., Haupt, A., Bildl, W., Boudkkazi, S., Roeper, J., Fakler, B., Schulz, U., 2014. Regional diversity and developmental dynamics of the AMPA-receptor proteome in the mammalian brain. *Neuron* 84, 41–54.
- Seelaar, H., Rohrer, J.D., Pijnenburg, Y.A., Fox, N.C., van Swieten, J.C., 2011. Clinical, genetic and pathological heterogeneity of frontotemporal dementia: a review. *J. Neurol. Neurosurg. Psychiatry* 82, 476–486.

- Shi, S., Hayashi, Y., Esteban, J.A., Malinow, R., 2001. Subunit-specific rules governing AMPA receptor trafficking to synapses in hippocampal pyramidal neurons. *Cell* 105, 331–343.
- Song, R.S., Tolentino, R., Sobie, E.A., Neves-Zaph, S.R., 2016. Cross-regulation of phosphodiesterase 1 and phosphodiesterase 2 activities controls dopamine-mediated striatal  $\alpha$ -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor trafficking. *J. Biol. Chem.* 291, 23257–23267.
- Stevens, M., van Duijn, C.M., Kamphorst, W., de Knijff, P., Heutink, P., van Gool, W.A., Scheltens, P., Ravid, R., Oostra, B.A., Niermeijer, M.F., van Swieten, J.C., 1998. Familial aggregation in frontotemporal dementia. *Neurology* 50, 1541–1545.
- Summa, M., Di Prisco, S., Grilli, M., Marchi, M., Pittaluga, A., 2011. Hippocampal AMPA autoreceptors positively coupled to NMDA autoreceptors traffic in a constitutive manner and undergo adaptative changes following enriched environment training. *Neuropharmacology* 61, 1282–1290.
- Terashima, A., Pelkey, K.A., Rah, J.C., Suh, Y.H., Roche, K.W., Collingridge, G.L., McBain, C.J., Isaac, J.T., 2008. An essential role for PICK1 in NMDA receptor-dependent bidirectional synaptic plasticity. *Neuron* 57, 872–882.
- Udagawa, T., Fujioka, Y., Tanaka, M., Honda, D., Yokoi, S., Riku, Y., Ibi, D., Nagai, T., Yamada, K., Watanabe, H., Katsuno, M., Inada, T., Ohno, K., Sokabe, M., Okado, H., Ishigaki, S., Sobue, G., 2015. FUS regulates AMPA receptor function and FTLD/ALS-associated behaviour via GluA1 mRNA stabilization. *Nat. Commun.* 6, 7098.
- Ziemann, U., Rothwell, J.C., Ridding, M.C., 1996. Interaction between intracortical inhibition and facilitation in human motor cortex. *J. Physiol.* 496, 873–881.