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ORIGINAL ARTICLE

# Changes of several brain receptor complexes in the cerebral cortex of patients with Alzheimer disease: probable new potential pharmaceutical targets

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**Abstract** Although Alzheimer disease (AD) has been linked to defects in major brain receptors, studies thus far have been limited to the determination of receptor subunits or specific ligand binding studies. However, the availability of current technology enables the determination and quantification of brain receptor complexes. Thus, we examined levels of native receptor complexes in the brains of patients with AD. Cortical tissue was obtained from control subjects ($n = 12$ females and 12 males) and patients with AD ($n = 12$ females and 12 males) within a 3-h postmortem time period. The tissues were kept frozen until further biochemical analyses. Membrane proteins were extracted and subsequently enriched by ultracentrifugation using a sucrose gradient. Membrane proteins were then electrophoresed onto native gels and immunoblotted using antibodies against individual brain receptors. We found that the levels were comparable for complexes containing GluR2, GluR3 and GluR4 as well as 5-HT$1_A$. Moreover, the levels of complexes containing muscarinic AChR M1, NR1 and GluR1 were significantly increased in male patients with AD. Nicotinic AChRs 4 and 7 as well as dopaminergic receptors D1 and D2 were also increased in males and females with AD. These findings reveal a pattern of altered receptor complex levels that may contribute to the deterioration of the concerted activity of these receptors and thus result in cognitive deficits observed in patients with AD. It should be emphasised that receptor complexes function as working units rather than individual subunits. Thus, the receptor deficits identified may be relevant for the design of experimental therapies. Therefore, specific pharmacological modulation of these receptors is within the pharmaceutical repertoire.

**Keywords** Alzheimer disease · Cerebral cortex · Receptors · Receptor complexes · Dopamine receptor · Acetylcholine receptor

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## Introduction

Alzheimer disease (AD) is the most common form of dementia found in elderly persons and is a neurodegenerative disease that is marked by a decline in memory and cognitive performance, including the deterioration of language and defects in visual and motor coordination, which results in eventual death (Cummings 2004). Alzheimer disease is morphologically characterised by extracellular beta-amyloid (Aβ) plaque deposition, intraneuronal tau pathology, neuronal cell death (which begins in the entorhinal cortex and later spreads to the neocortex), a loss of specific receptor types, (Bernareggi et al. 2007; Braak et al. 2006) and vascular dysfunction and inflammatory processes (DeCarli 2003; Petersen et al. 2001; Ghidoni et al. 2013; Grammas 2011).

Several lines of evidence have indicated a link between brain nicotinic acetylcholine receptors (nAChRs) (Buckingham et al. 2009) and AD development. Biochemical analysis of the brains of AD patients revealed cholinergic deficits, i.e., an increase in butyrylcholinesterase, reduction in acetylcholine and attenuation of cholinergic synthetic [choline acetyltransferase (ChAT)] and inactivating [acetyl choline esterase; (AChE)] enzymatic activity (Bartus et al. 1982; Francis et al. 1999). The most vulnerable neurons in AD appear to be those that express high levels of nAChRs, particularly neurons that contain the α7 subunit (D'Andrea and Nagele 2006) of the nAChR. Moreover, several associated proteins were also down-regulated in AD patients (Martin-Ruiz et al. 1999; Gotti et al. 2006; Sabbagh et al. 2006). In addition, α7 nAChRs have been found to colocalise with plaques, (Wang et al. 2000) and the α7 and α4 subunits have also been positively associated with neurons that accumulate Aβ (Wevers et al. 1999).

The discovery that nicotine, which is a ligand for nAChRs, and its mimetics are neuroprotective against Aβ toxicity (Kihara et al. 1998) is of interest, particularly in view of the observation that nicotine also enhances cognition (Rusted et al. 2000). Nicotinic AChRs play a prominent role in nicotinic protection that is blocked by the nicotinic antagonists, dihydro-β-erythroidine and mecamylamin (Kihara et al. 2001; Takada-Takatori et al. 2006). Additionally, donepezil and rivastigmine (commercially known as Aricept and Exelon, respectively) are acetylcholinesterase (AChE) inhibitors that are currently used in treatments for mild or moderate AD and can also protect cultured neuroblastoma cells against toxic effects of Aβ (Buckingham et al. 2009).

There is growing evidence that cholinesterase inhibitors promote the release of glutamate from pyramidal neurons, which potentially increases cortical ACh levels and the subsequent activation of AChRs. In addition, it has been demonstrated that uncoupling of the postsynaptic muscarinic M1 receptor from G-proteins is associated with the loss of *N*-methyl-D-aspartate (NMDA) receptor density and protein kinase C (PKC) activity in the postmortem frontal cortices of individuals with AD (Revett et al. 2012).

A selective serotonin 1A receptor (5-HT$1_A$R) molecular imaging probe was used in combination with positron emission tomography to quantify the 5-HT$1_A$R density in the brains of AD patients with mild cognitive impairment and control individuals. Alzheimer disease patients' brains showed a significant decrease in receptor densities in the hippocampi and Raphe nuclei (Kepe et al. 2006). Furthermore, glutamatergic neurons are located in areas that are known to be affected in AD, and this initial damage has been proposed to initiate in pyramidal neurons in layers III and V of the neocortex (Kowall and Beal 1991; Bussiere et al. 2003) as well as in glutamatergically innervated cortical and hippocampal neurons (Revett et al. 2012; Francis 2003).

Although it is known that AD is a synaptic disease that involves various neurotransmitter systems, particularly those involving acetylcholine or glutamate, very little is known regarding the qualitative and quantitative changes in neurotransmitter receptors in the AD brain. Previously, it was shown that cell membranes carrying neurotransmitter receptors in the human postmortem brain retain their function upon transplantation into frog oocytes. Bernareggi et al., therefore, examined the properties of glutamate receptors (GluRs) in the cerebral cortices of AD and non-AD brains and found that oocytes injected with AD membranes acquired GluRs that exhibited the same functional properties as oocytes injected with membranes from non-AD brains. However, the amplitudes of the currents elicited by the GluRs were smaller in oocytes injected with membranes from AD brains. Western blotting analyses of the same membrane preparations used for the electrophysiological studies also showed that AD membranes contained a significantly lower amount of GluR2/3 subunit proteins. Furthermore, the corresponding mRNA levels were also decreased in the AD brain. Thus, the smaller amplitude of membrane currents elicited by glutamate in oocytes injected with membranes from the AD brain was likely a consequence of the reduced number of GluRs in cell membranes transplanted from the AD brain (Bernareggi et al. 2007).

Previous studies on brain receptor subunits have also shown the importance of these signalling compounds in AD, although there is currently no evidence for changes in the receptor complexes. Using a native gel-based immunohistochemical approach to determine the levels of major brain receptor complexes in AD patient brains, we revealed that a series of changes in the levels of receptor complexes in the cortical brain areas of AD patients and control subjects resulted in (or occurred concomitantly with) the deterioration of the physiological concerted activity of the receptor complexes.

## Materials and methods

### Patients

#### *Case recruitment and characterization of human brain tissue*

Case recruitment and autopsy were performed in accordance with guidelines effective at the Banner Sun Health Research Institute Brain Donation Program of Sun City, Arizona, USA (Beach et al. 2008). The required consent was obtained for all cases. The definite diagnosis of AD for

all cases used in this study was based on the presence of neurofibrillary tangles and neuritic plaques in the hippocampal formation and neocortical areas and met the criteria of the National Institute on Aging (NIA) and the Consortium to Establish a Registry for AD (CERAD), (Mirra et al. 1991). Cortical tissue from 24 AD cases (12 female, 12 male) and from 24 age-matched control subjects (12 female, 12 male) was evaluated for neurotransmitter receptor complexes (Supplemental Table 1).

## Biochemical analyses

### *Sample preparation*

Forty-eight cortical samples obtained from AD patients and control subjects (12 male and 12 female controls as well as 12 male and 12 female patients with AD) were homogenised in ice-cold homogenisation buffer [10 mM HEPES, pH 7.5, 300 mM sucrose, one complete protease inhibitor tablet (Roche Molecular Biochemicals, Mannheim, Germany) per 50 mL] by Ultra-Turrax (IKA, Staufen, Germany). The homogenate was then centrifuged for 10 min at 1,000×*g* and the pellet was discarded. The supernatant was centrifuged at 50,000×*g* for 30 min in an ultracentrifuge (Beckman Coulter Optima L-90 K), and the resulting pellet was homogenised in 5 mL of washing buffer (homogenisation buffer without sucrose), kept on ice for 30 min and centrifuged at 50,000×*g* for an additional 30 min.

### *Sucrose gradient ultracentrifugation for membrane fractionation*

The plasma membrane purification procedures for the pellet was performed as previously described with slight modifications (Chen et al. 2006; Kang et al. 2008). Sucrose density gradient centrifugation solutions (700 µL) with sucrose contents of 69, 54, 45, 41 and 37 % (w/v) were used. Membrane pellets (500 µL) were resuspended in homogenisation buffer and were layered on top of tubes that were filled with homogenisation buffer. The samples were then ultra centrifuged at 4 °C at 70,000×*g* for 3 h. After centrifugation, the 41 % fraction from the sucrose interface was collected, diluted 10 times with homogenisation buffer and then ultracentrifuged at 4 °C at 100,000×*g* for 30 min. After the supernatant was discarded, the pellet was stored at −80 °C until further analysis (Ghafari et al. 2012a).

### *Blue native-polyacrylamide gel electrophoresis (BN-PAGE)*

Membrane pellets from the 41 % sucrose gradient ultracentrifugation fraction were solubilised in extraction buffer (1.5 M 6-aminocaproic acid, 300 mM Bis–Tris, pH 7.0) and 10 % Triton X-100 (stock solution was added at a ratio of 1:4 to achieve a final concentration of 2 % Triton X-100) and vortexed every 10 min for 1 h. Following solubilisation, the samples were cleared using centrifugation at 20,000×*g* for 60 min at 4 °C (Supplemental Method 1a) (Ghafari et al. 2012a). Native high-molecular-mass markers were purchased from Invitrogen (Carlsbad, CA, USA).

### *Immunoblotting*

Membrane proteins were transferred from BN-PAGE to PVDF membranes. After the membranes were blocked for 1 h with 10 % nonfat dry milk in 0.1 % TBST (100 mM Tris–HCl, 150 mM NaCl, pH 7.5, 0.1 % Tween 20), the membranes were incubated in the following primary antibodies: rabbit anti-GluR1 (Abcam, ab31232, Cambridge, UK, 1/5,000), rabbit anti-GluR2 (Abcam, ab52932, Cambridge, UK, 1/5,000), rabbit anti-GluR3 (Abcam, ab87609, Cambridge, UK, 1/5,000), rabbit anti-GluR4 (Abcam, ab109431, Cambridge, UK, 1/3,000), rabbit anti-dopamine receptor D1 (Abcam, ab85608, Cambridge, UK, 1/2,500), rabbit anti-dopamine receptor D2 (Millipore, AB5084P, 1/3,000), rabbit anti-nicotinic acetylcholine receptor alpha4 (Abcam, ab41172, Cambridge, UK, 1/3,000), rabbit anti-nicotinic acetylcholine receptor alpha7 (Abcam, ab10096, Cambridge, UK, 1/3,000), rabbit anti-muscarinic receptor M1 (Abcam, ab75178, Cambridge, UK, 1/4,000), rabbit anti-NMDAR1 (Abcam, ab28669, Cambridge, UK, 1/2,500) and rabbit anti-5HT1$_A$ (GenScript, Piscataway, NJ, USA, 1/20,000). The antibodies were detected using horseradish peroxidase-conjugated anti-rabbit IgG (Abcam, ab6721, Cambridge, UK, 1/10,000), and the membranes were developed using the ECL Plus Western Blotting Detection System (GE Healthcare, Buckinghamshire, UK). The arbitrary optical density of the immunoreactive bands was measured using ImageJ software (http://rsb.info.nih.gov/ij/).

### *Immunoprecipitation of the dopamine D1 receptor in human brains*

To identify the interacting partners of the D1 receptor, immunoprecipitation studies were performed using cortical human brain tissue.

Total membrane fractions (Ghafari et al. 2012a) were obtained from the Cortical tissue and suspended in lysis buffer containing 1 % Triton X100, 150 mM NaCl, 1 mM EDTA, 50 mM Tris–HCl (pH 8.0), 10 mM NaF, 10 mM $Na_3VO_4$ and protease inhibitor cocktail (Roche, Mannheim, Germany) and placed on a rotation shaker for 1 h at 4 °C. After centrifugation at 15,300×*g* and 4 °C for 10 min, the supernatant was incubated with affinity-purified rabbit antibody against the dopamine D1 receptor

(D1DR, Santa Cruz, Santa Cruz, CA, USA). Next, the sample was incubated with protein G agarose beads (GE Healthcare, Uppsala, Sweden) for 4 h at 4 °C with gentle rotation. After five washes with the same lysis buffer, the bound proteins were denatured in sample buffer containing 125 mM Tris (pH 6.8), 4 % SDS, 20 % glycerol, 10 % beta-mercaptoethanol and 0.02 % bromophenol blue at 95 °C for 3 min (Ghafari et al. 2012b). The samples were then loaded onto 10 % SDS–polyacrylamide gels, electrophoresed and subsequently transferred onto PVDF membranes (Pall, Ann Arbor, MI, USA). After the membranes were blocked for 1 h in 5 % nonfat dry milk in 0.1 % TBST (100 mM Tris–HCl, 150 mM NaCl, pH 7.5 and 0.1 % Tween 20), the membranes were incubated with a diluted goat primary antibody against the dopamine D1 receptor (41:1,000, Santa Cruz, Santa Cruz, CA, USA) and detected using anti-goat IgG (1:5,000, Abcam, Cambridge, UK). The membranes were then developed using an ECL Plus Western Blotting Detection System (GE Healthcare, Uppsala, Sweden).

*In-gel digestion of proteins and peptides*

Spots selected from the SDS gel that were recognised by antibodies against the dopamine D1 receptor subunit were placed into a 1.5-mL Eppendorf tube, and in-gel digestion was performed as previously described (Supplemental Method 1b), (Ghafari et al. 2012b).

Mass spectrometry

The samples were analysed by injecting 15 µl into a Dionex 3000 nano-LC system equipped with C18 Acclaim Pepmap100 columns (Thermo Fisher Scientific, Austria) and then loading onto a precolumn for 10 min at a flow rate of 10 µl/min using 100 % mobile phase A. The samples were then separated at a flow rate of 300 nl/min using a linear gradient from 7 to 40 % mobile phase B for 45 min starting at 1 min after valve switching, followed by 10 min of equilibration with a total run time of 66 min (mobile phase A: 2 % ACN, 0.2 % formic acid; mobile phase B: 80 % ACN, 0.2 % formic acid; all solvents were of LC–MS grade). The analytes were then ionised using a nanospray ion source (Thermo Fisher Scientific, Austria), and MS analysis was performed on a QExactive Orbitrap (Thermo Fisher Scientific, Austria). The peptides were detected over a scan range from 400 to 1,400 Th with a target mass resolution of $R = 70{,}000$ at m/z 200. The instrument was operated in DDA mode with MS/MS scans of the top six precursor ions from the preceding MS scan in the HCD trap of the instrument (normalised collision energy set to 30 %, resolution set to 17,500) (Groessl et al. 2012).

MASCOT searches were performed using MASCOT 2.2.06 (Matrix Science, London, UK) against the latest UniProtKB database for protein identification and the Modiro® search engine using parameters as previously described (Supplemental Method 1c), (Ghafari et al. 2012b).

Statistical evaluation

Data obtained from Western blotting were analysed using Student's $t$ test and presented as the mean ± SD. Calculations and Pearson correlations of the data were performed using SPSS for Windows 19.0.

To determine whether the receptor complexes consisted of individual subunits, a comparative blot was performed.

## Results

The receptor complex levels were comparable for complexes consisting of GluR2, GluR3, GluR4 and 5-HT1$_A$ (Supplemental Figs. 1–4). In addition, the mobilities of the complexes were also comparable. This finding indicates that receptor complexes consisting of the GluR2–4 and 5-HT1A subunits were qualitatively and quantitatively unchanged.

The amount of receptor complexes containing muscarinic AChR M1, NR1 and GluR1 was significantly increased in male patients with AD (Fig. 1). No changes in electrophoretic mobility were observed in either group.

A single band was observed between 480 and 720 kDa for M1, between 480 and 720 kDa for NR1 (but with a lower mobility than that of M1) and between 480 and 720 kDa with the same mobility as GluR2–4, but different from that of M1 and NR1.

The amount of these three receptor complexes was significantly increased in male patients with AD compared with control individuals. The electrophoretic pattern of mobility was also comparable between the groups.

The levels of the receptor complexes containing nicotinic AChR 4 and 7 were increased in both sexes (Fig. 2a). The receptor complexes of nAChR4 and 7 co-migrated to the same position, which was between 480 and 720 kDa. The electrophoretic mobility was also comparable between the groups.

The amount of receptor complexes containing the dopaminergic receptors D1 and D2 was increased in males and females with AD, the complexes and migrated between 480 and 720 kDa. However, these receptor complexes demonstrated differing mobility (Fig. 2b). Both receptor complexes showed comparable electrophoretic mobility in control subjects and patients with AD.

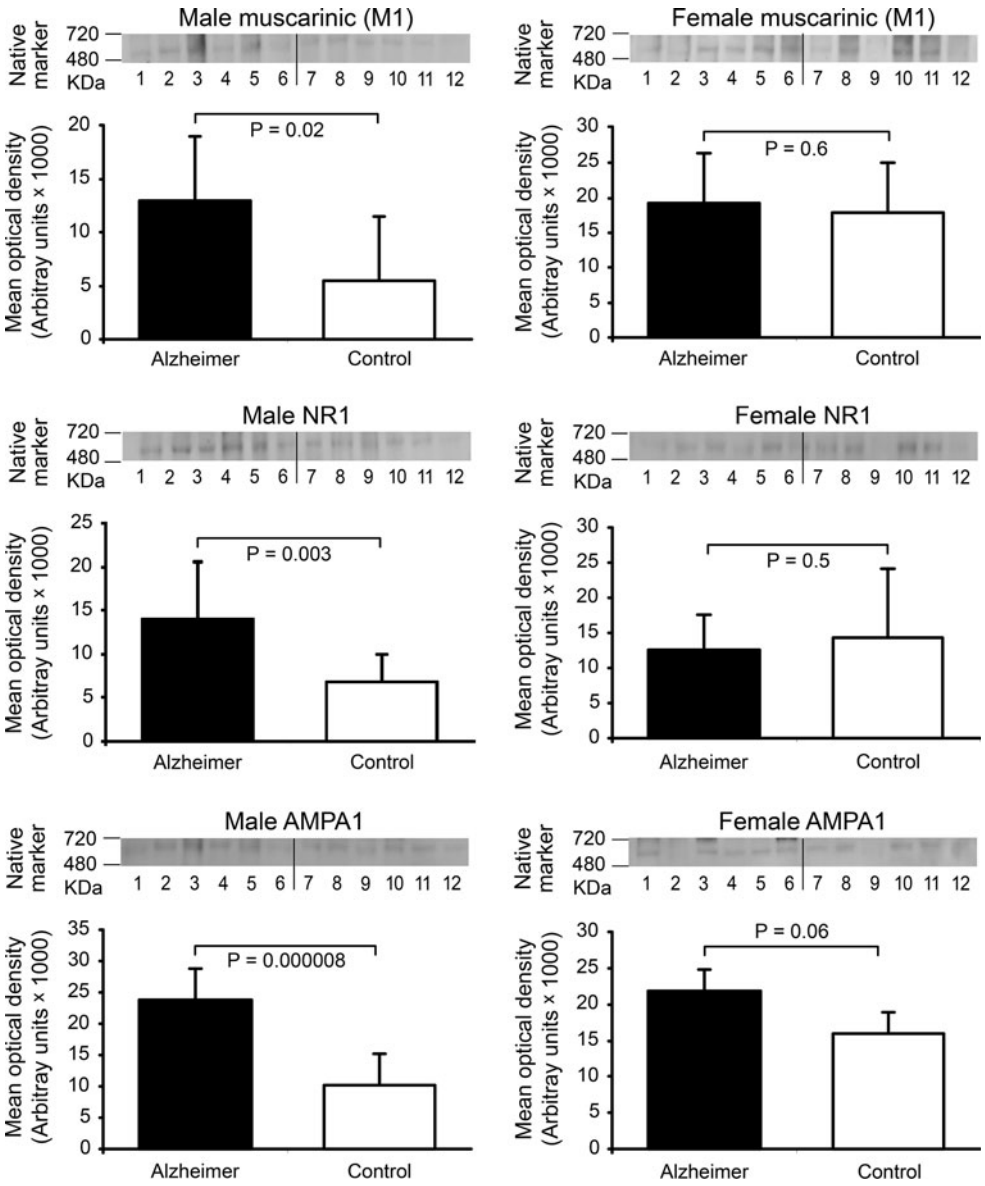


**Fig. 1** Receptor complex levels differ between male control subjects and male patients with AD. The receptor complex levels are expressed as arbitrary units of optical density

A blot comparing the mobility of receptor complexes consisting of the individual subunits is shown in Fig. 3.

Receptor complexes consisting of the AChR subunit M1 showed a mobility that was different from that of all of the other receptor complexes, but comparable to that of complexes containing nAChR 4 and 7. NR1-containing receptor complexes migrated to a position that differed from that of complexes containing nAChR 4 and 7. All of the AMPA receptor subunit (GluR1–4)-containing complexes were observed at an identical electrophoretic position, which was different from the pattern observed for other receptor complexes. Moreover, D1- and D2-containing complexes showed comparable mobility that differed from the mobility of all of the other receptor complexes. Furthermore, 5HT1A-containing receptor complexes showed individual and different mobilities that were not comparable to those of the other receptor complexes.

A series of receptor complexes was correlated in control subjects as well as in patients with AD. However, the correlation patterns were different between the control subjects and patients with AD, which most likely indicates a deterioration of the concerted activity of the receptor complexes.

Moreover, receptor complexes in males and females from the control group showed a different pattern of association (Table 1a, b; Supplemental Tables 2a, 2b).

In addition, receptors in male and female patients with AD showed correlation patterns that differed from those of the control group (Table 2a, b; Supplemental Tables 3a, 3b).

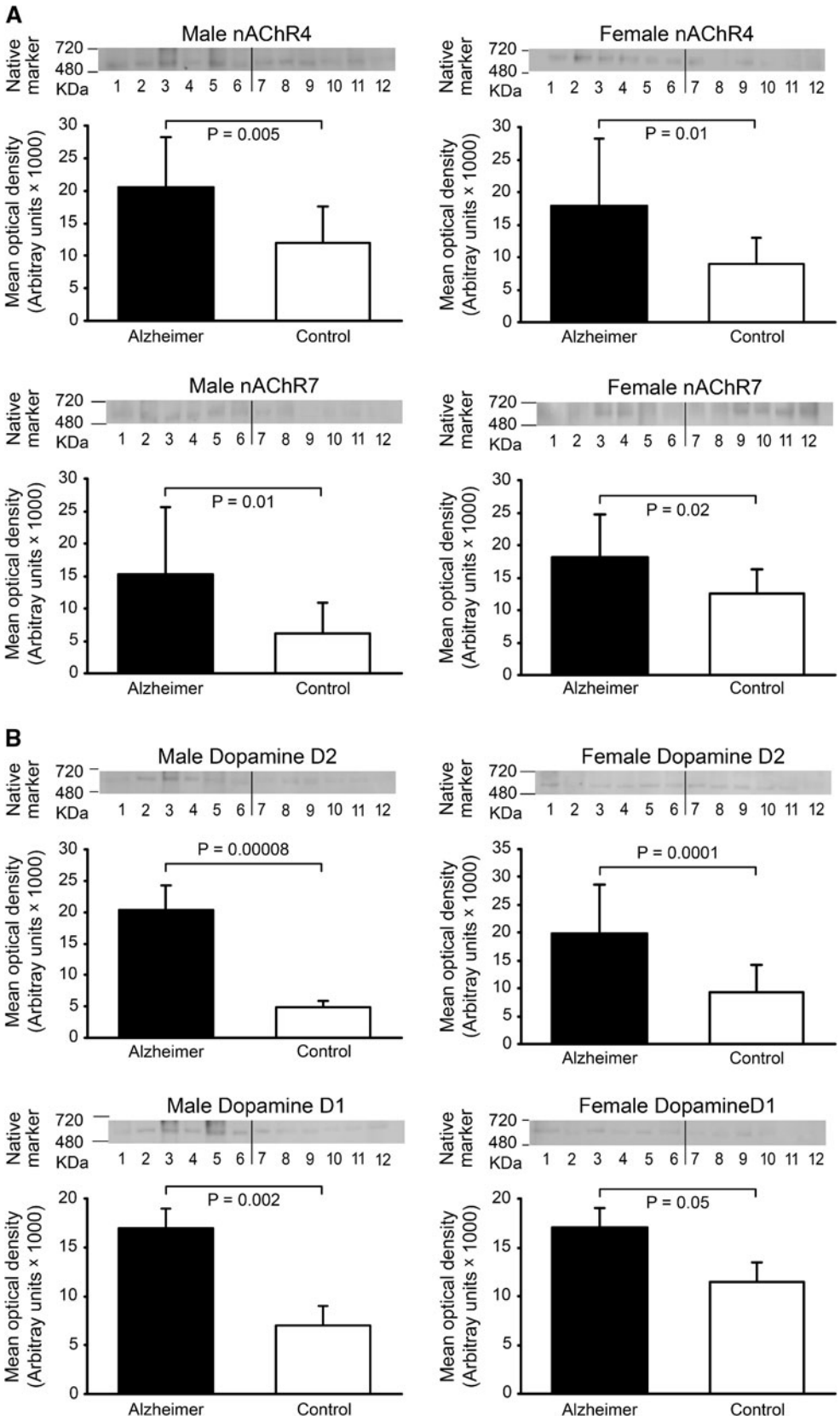


**Fig. 2** Receptor complex levels differ between both males and females in control subjects and patients with AD

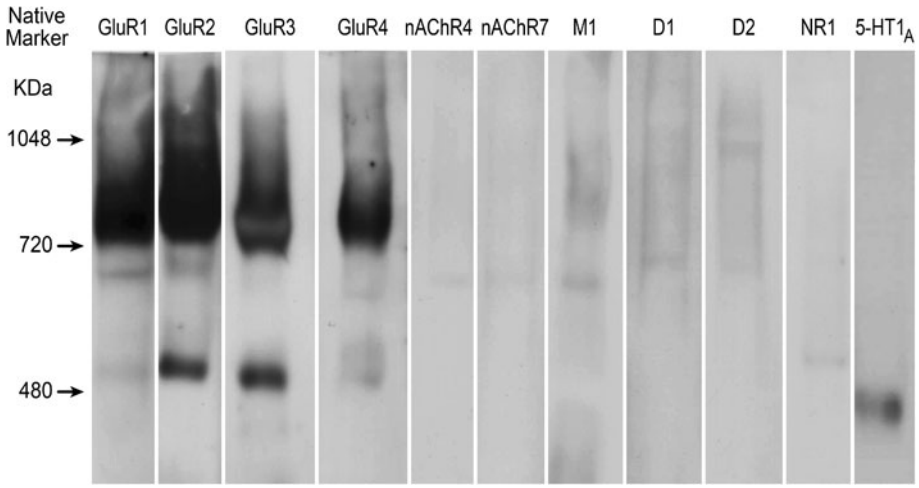


**Fig. 3** Alignment of receptor complexes to determine the receptor complex composition. The high-molecular-weight material was produced by glycosylation and is known to generate this specific pattern, which was not included in the quantification

**Table 1** Correlation between receptor complexes in males and females from the control group

| Receptor complexes | Correlation | | | | | |
|---|---|---|---|---|---|---|
| | GluR3 | nAChR4 | nAChR7 | NR1 | M1 | 5-HT1$_A$ |
| Male control | | | | | | |
| GluR1 | | | | 0.04* | | 0.012* |
| GluR2 | 0.010** | 0.006** | | | | |
| GluR3 | | | | | | 0.04* |
| D1 | | | 0.002** | | 0.000** | 0.001** |
| nAChR7 | | | | | 0.000** | 0.005** |
| NR1 | | | | | | 0.04* |
| M1 | | | 0.000** | | | 0.01* |
| Receptor complexes | Correlation | | | | | |
| | D1 | D2 | nAChR4 | nAChR7 | NR1 | M1 |
| Female control | | | | | | |
| GluR2 | 0.02* | 0.006** | | 0.05* | | |
| GluR4 | | | 0.006** | | 0.01* | 0.009** |
| nAChR4 | | | | | 0.007** | |

ns $P > 0.05$
* $P \leq 0.05$
** $P \leq 0.01$
*** $P \leq 0.001$
**** $P \leq 0.0001$

Binding partners indicating the presence of several receptor subtypes in the D1 receptor complexes are shown in Supplemental Fig. 5, as determined using immunoprecipitation followed by mass spectrometrical identification. The representative mass spectrometric spectra are shown in Supplemental Fig. 6.

The list of peptides identified and information on sequence coverage are provided in Supplemental Tables 4 and 5, respectively.

Sex differences between the receptor complexes are shown in Figs. 1, 2 and Supplemental Figs. 1–4.

## Discussion

The major outcome of the study was the identification of sex-dependent alterations of receptor complexes containing NR1, GluR1, mAChR M1 and nAChR 4 and 7 as well as

**Table 2** Correlation between receptor complexes in males and females from the Alzheimer group

| Receptor complexes | Correlation | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | GluR1 | GluR3 | D1 | D2 | nAChR4 | nAChR7 | M1 | 5-HT1$_A$ |
| Male Alzheimer | | | | | | | | |
| GluR1 | | 0.02* | 0.003** | 0.000** | 0.007** | 0.02* | 0.001** | |
| GluR2 | | | | | | | | 0.005** |
| GluR3 | 0.02* | | | | | | | |
| GluR4 | | | | | 0.005** | | 0.02* | |
| D1 | 0.003** | | | 0.009** | 0.01* | | 0.007** | |
| D2 | 0.000** | | 0.009** | | 0.002** | | 0.000** | |
| nAChR4 | 0.007** | | 0.01* | 0.002** | | | 0.002** | |

| Receptor complexes | Correlation | | | | | | |
|---|---|---|---|---|---|---|---|
| | GluR1 | GluR4 | D1 | D2 | nAChR4 | nAChR7 | M1 |
| Female Alzheimer | | | | | | | |
| GluR1 | | 0.01* | 0.03 | | 0.02* | | |
| GluR2 | | | 0.003** | 0.01* | | | |
| GluR3 | | | | | | 0.02* | 0.04* |
| GluR4 | 0.01* | | | 0.03* | | | |
| D1 | 0.03* | | | 0.01* | | | |
| D2 | | 0.03* | 0.01* | | | | |

ns $P > 0.05$

* $P \leq 0.05$

** $P \leq 0.01$

*** $P \leq 0.001$

**** $P \leq 0.0001$

D1 and D2 in cortical samples of patients with AD. The receptor complex levels differed between men and women in both the control subjects and patients with AD. Moreover, a receptor network for the control population and AD cohort was established based on patterns and correlations that reflected impaired brain wiring at the receptor level, as well as the loss of concerted activity in the control cohort. This study was performed at the lowest possible post-mortem period of 3 h.

As previously described, both nACh receptors and mACh receptors are involved in learning and memory and particularly in the disease mechanisms of AD. Thus, M1 complex levels were increased in cortical samples of male, but not female, patients with AD. This finding has never been previously reported and should be considered as a potential biomarker or pharmacological target in the male AD population. However, it may also simply reflect the complex deterioration of the concerted activity of brain receptors in this disorder as well as the interaction between M1 and NMDA receptors that has previously been proposed at the receptor subunit level. Moreover, nAChR 4 and 7 receptor complex levels were increased in both male and female AD patients. A potential link between nAChR 4 and 7 and AD has been previously reported, where colocalisation of these receptor subunits was observed in AD patient brain (Wevers et al. 1999). However, there has been no report regarding the nAChR complex levels.

Taken together, these findings may contribute to the understanding of mechanisms underlying the pathobiochemistry of AD, extend the repertoire of known potential biomarkers of AD and provide insight into the pharmacological modulation of the ACh system for experimental therapies.

Thus far, no direct interactions among M1, nACh4 and nACh7 have been found with other brain receptors. Furthermore, the comparable electrophoretic mobility among nACh4, nACh7 and M1 may indicate the presence of these subunits in the complexes observed (Fig. 3). Interactions between 5HT-1$_A$R and nACh7 have been previously reported (Pandya and Yakel 2013); however, alterations in nAChR levels were not accompanied by changes in 5HT-1$_A$R complexes in this study. Furthermore, there is no evidence for the presence of 5HT1$_A$Rs in nACR complexes (Fig. 3).

Although the contribution of NMDARs has been well studied in AD (Xu et al. 2012), there is a lack of

information regarding NR1 receptor complexes in the AD brain. In males, NR1 receptor complex levels were significantly elevated, which indicates the involvement of these NR1-containing complexes in AD. Moreover, NR1 is required for cognitive functions including learning and memory, and dysregulation of NR1 may reflect memory deficits in male patients with AD. However, this change may also arise from the deterioration of the orchestrated function of other receptor complexes.

Of the four AMPAR complexes tested, the amount of GluR1 complexes was significantly increased in the brains of male AD patients. This was consistent with increased levels of NR1, which regularly interacts with AMPA receptors. Moreover, AMPAR deficits have a well-known role in cognitive function and AD (Chang et al. 2012). We consider the observed changes in D1 and D2 receptor complex levels to be our most intriguing novel findings. The levels of both receptor complexes were increased in AD in both males and females. Neurotransmission via G protein-coupled dopamine receptors plays a key role in many forms of learning and memory; D1 and D2 receptors have been shown to contribute to the establishment of several forms of memory that are mediated by the cortex (Rinaldi et al. 2007; Vijayraghavan et al. 2007). In addition, D2 and D1 receptors are critical for the formation (Guarraci et al. 2000) and extinction (El-Ghundi et al. 2001) of fear memory in the amygdala, respectively, and the D1 and D5 receptors are required for motor-skill learning in the striatum (Willuhn and Steiner 2008). At the cellular level, D1 receptors can enhance NMDA receptor-dependent LTP in the prefrontal cortex via activation of protein kinase A (PKA), (Gurden et al. 2000) and the D1 family of receptors plays a critical role in both LTP and LTD at corticostriatal synapses (Ng et al. 2010; Calabresi et al. 2007). Furthermore, there is broad evidence for the interaction of D1 and D2 receptors with other brain receptor systems; there is a close interaction between brain glutamatergic NMDA receptors and the monoamine dopaminergic system (de Bartolomeis et al. 2005). Dopaminergic disruptions in the brain can result in glutamatergic NMDA receptor changes (Hallett et al. 2006) and vice versa (Hallett and Standaert 2004). NMDA receptors also mediate dopaminergic–glutamatergic interactions by the direct coupling of both NR1 and NR2A subunits with the C terminus of the dopamine D1 receptor, and dopaminergic depletion can change the levels of NMDA receptor expression (Hallett and Standaert 2004; Xu et al. 2012). In the current study, we performed immunoprecipitation followed by mass spectrometry to identify binding partners for the receptor complexes. We found that D1 is a component of a complex with NR1, GluR1, 2 and 4 (Supplemental Fig. 5). Furthermore, in male patients with AD, levels of the NR1 complex were increased.

In addition, D1/D5 receptor activation also resulted in increased cellular expression of GluR1 and its incorporation at synaptic sites (Smith et al. 2005). Activation of dopaminergic D1 receptors in hippocampal neurons increased the pool of extrasynaptic, but not synaptic AMPARs, through a PKA-dependent mechanism, and their subsequent incorporation into synapses required CaMKII activity (Gao et al. 2006). Brief stimulation of D1 dopaminergic receptors, which facilitates LTP in neurons of the prefrontal cortex through a PKA-dependent mechanism, results in the clustering of GluR1-containing AMPARs near, but not within, the PSD (Sun et al. 2005; Derkach et al. 2007). In this study, the amount of GluR1-containing complexes was increased in male patients with AD, which is consistent with previous described interactions.

## Conclusion

We have demonstrated a network of brain receptor complexes for a control population and a changed network for cortical samples obtained from AD patients, as shown by associations between receptor systems and receptor complex patterns. Consistent with the literature, nicotinergic and muscarinic cholinergic abnormalities were both observed. Several brain receptor types were also unchanged between the control subjects and AD patients, and changes in the dopaminergic receptor system are a novel and intriguing finding that warrants further investigation. The D1 complex was characterised by immunoprecipitation and included NMDAR and AMPAR components. This study not only provides evidence and a basis for further studies, but may also provide insight into previous studies on receptors in AD and enable the design of future studies in AD at the level of receptor complexes because receptor complexes, rather than receptor subunits, may play an important role in mechanisms of AD and other degenerating diseases.

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**Conflict of interest** The authors declare that there is no conflict of interest.

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