Cortical Plasticity in Alzheimer's Disease in Humans and Rodents

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Background: The aim of this study was to determine whether neocortical long-term potentiation (LTP) is deficient in patients with Alzheimer's disease (AD) and in amyloid precursor protein (APP)/presenilin-1 (PS1) mice, an AD animal model. We then ascertained whether this deficit might be paralleled by functional abnormalities of N-methyl-D-aspartate (NMDAR) glutamate receptors.

Methods: We studied neocortical LTP-like plasticity in 10 patients with mild-to-moderate AD and 10 age-matched normal controls using paired associative stimulation (PAS). We assessed neocortical (medial prefrontal cortex and primary motor cortex) and hippocampal LTP in brain slices of symptomatic APP/PS1 mice. NMDAR composition and signaling as well as synaptic calcium influx were determined in motor, prefrontal and hippocampal cortices of APP/PS1 mice.

Results: Both AD patients and transgenic animals showed a deficit in NMDAR-dependent forms of neocortical plasticity. Biochemical analysis showed impaired NMDAR function in symptomatic APP/PS1 mice.

Conclusions: Neocortical plasticity is impaired in both patients with AD and APP/PS1 mice. The results of our biochemical studies point to impaired NMDAR function as the most likely cause for the neocortical plasticity deficit in AD.

Key Words: Alzheimer's disease, glutamate, LTP, TMS

Alzheimer's disease (AD) is a neurodegenerative disorder in which progressive memory loss is accompanied by cognitive decline. Although abnormalities are more prominent in the temporal lobe, increasing clinical and instrumental evidence also points to diffuse neocortical involvement (1,2). Based upon recent findings, synaptic dysfunctions and abnormal neuroplasticity are becoming new and attractive hypotheses to explain, at least in part, the pathogenesis of AD. For instance, it is well established that hippocampal long-term potentiation (LTP), a molecular correlate of synaptic plasticity, is impaired in different transgenic AD models (3). In particular, mice overexpressing amyloid precursor protein (APP) (K670N:M671L) together with presenilin-1 (PS1) (M146L) display reduced LTP as early as at three months of age, a phenomenon that is paralleled by plaques appearance, increased Aβ levels and working memory abnormalities (4). Glutamate signaling through postsynaptic N-methyl-D-aspartate receptor (NMDAR) plays an important role in cortical LTP (5–7). It is conceivable that the prominent neurophatology in the cortical glutamatergic pyramidal neurons and their synaptic loss might also affect neocortical plasticity in AD.

Neocortical plasticity in humans can be investigated with paired associative stimulation (PAS) (Figure 1A), a noninvasive cortical stimulation paradigm, that induces long-lasting LTP-like increase in the cortical output circuits (8). This form of plasticity is NMDAR-dependent and follows Hebbian rules (9).

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In the present study, we employed PAS to examine neocortical synaptic plasticity in patients with mild-to-moderate AD. To investigate further the putative mechanisms that might underlie cortical plasticity impairment in AD, we studied NMDA-dependent forms of neocortical LTP and NMDAR composition and signaling in brain slices from 4–4.5 month old APP/PS1 mice. At this age APP/PS1 mice have only mild to-moderate cognitive impairment (4).

We thus used this age to grossly match the stage of AD patients. We hypothesize that in AD the prominent neurodegenerative processes in the neocortical glutamatergic system induce alteration of NMDAR signaling and NMDAR-dependent forms of cortical plasticity.

Methods and Materials

Patients' Experiment

Subjects. Ten patients with probable AD (Table 1) were recruited from the Dementia Unit, University of Messina, Italy and fulfilled the following inclusion criteria:

1) Probable AD according to National Institute of Neurological and Communicative Diseases and Stroke/Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) (10) and the DSM-IV (11);
2) mild-to-moderate AD as evidenced by Mini-Mental State Exam (MMSE) score of 12–26 (12);
3) No treatment with psychoactive drugs, including anticholinesterase inhibitors or *N*-methyl-D-aspartate antagonist or discontinuation at least 2 weeks before cognitive assessments;
4) Negative history for vascular dementia, stroke, epilepsy, convulsions;
5) Negative history for depression (score < 16 for Montgomery-Asberg Depression Rating Scale [MADRS]) (13);
6) Stable regimen for at least 12 months for females requiring estrogen replacement therapy for menopause;
7) Normal values for B12, folate, thyroid-stimulating hormone (TSH) and free-thyroxine (FT4).

On enrollment, all patients also underwent Alzheimer's Disease Assessment Scale-Cognitive Behavior (ADAS-cog) scale

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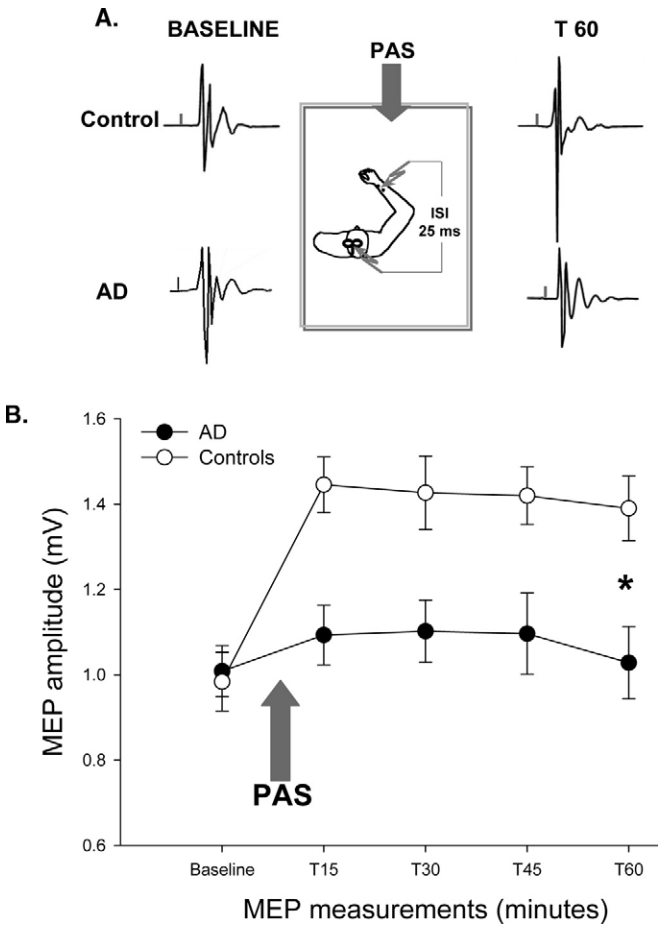

Figure 1. PAS induces MEP amplitude increase in normal controls but not in AD patients. **(A)** Data are shown for one control subject and one patient with AD at baseline and one hour after PAS (T60). At baseline MEP amplitude is the same in both AD patients and controls. Then, TMS pulse is applied to left M1 in close temporal relation to an electrical stimulus of the right median nerve at the wrist. If the stimuli are timed with an interstimulus interval of 25 msec, the sensory afferent input reaches the motor cortex just before the TMS is given. Repeated pairings (90 given every 3 sec) lead to long-lasting LTP like plasticity of the corticospinal system. Motor evoked potentials amplitudes were assessed at baseline and up to 1 hour after PAS (T15, T30, T45 and T60). At T60, one hour later, MEP amplitude increases in the control, but not in the AD patient. **(B)** Neocortical LTP-like plasticity. Each point represents the average of 10 subjects ± standard error. Data of controls are represented by empty circles, for patients by filled circles. PAS, paired associative stimulation; MEP, motor evoked potential; AD, Alzheimer's disease; TMS, transcranial magnetic stimulation; LTP, long-term potentiation.

(14). Magnetic resonance imaging (MRI) scans were also performed in all patients to exclude brain lesions such as infarcts, tumors, and normal pressure hydrocephalus.

Controls were 10 age-matched healthy caregivers (Table 1) without mood and cognitive disturbances (MMSE, ADAS-cog and MADRS scores within normal range). All participants were consistent right-handers according to the Edinburgh handedness inventory. All participants gave written informed consent according to the Declaration of Helsinki. The experimental protocol was approved by the local ethics committee (University of Messina, Italy).

Methods

Transcranial Magnetic Stimulation (TMS). Focal TMS of the left primary motor hand area was performed using a high-power Magstim 200 stimulator (Magstim, Whitland, Dyfed, Wales, United Kingdom) and a standard figure-of-eight coil, with external loop diameters of 9 cm. The coil was held tangentially to the skull with the handle pointing backwards and laterally at an angle of 45° to the sagittal plane. All subjects were seated in a comfortable reclining chair. EMG activity was recorded with two surface electrodes (Ag/AgCl) placed over the right abductor pollicis brevis (APB) muscle using a belly-tendon montage. EMG signals were amplified and filtered using a time constant of 3 msec and a high pass filter set a 3 kHz (Neurolog System, Digitimer Ltd., Welwyn Garden City, Herts, United Kingdom). Signals were acquired at a rate of 5 kHz (CED 1401 Laboratory Interface, Cambridge Electronic Design, Cambridge, United Kingdom) on a personal computer for off-line analysis. During the experiments electromyography (EMG) activity was continuously monitored with visual (oscilloscope) and auditory (speakers) feedback to ensure either complete relaxation at rest or a constant level of EMG activity during tonic contraction. We first determined resting motor threshold (RMT), defined as the minimal stimulus intensity required to produce motor evoked potentials (MEPs) > 50 μV in at least 5 out of 10 consecutive trials; then we studied MEP input-output curve at stimulus intensities ranging from 100 to 150% of the RMT.

Paired Associative Stimulation (PAS). AD patients and healthy subjects underwent to the protocol of PAS described by Stefan (8) for the induction of LTP-like M1 [primary motor cortex] plasticity (Figure 1A). PAS consisted of 200 electrical stimuli of the right median nerve at the wrist combined with suprathreshold magnetic pulse applied over the left primary motor hand area in the hot spot area (optimal for eliciting MEP in the right APB muscle). Mixed electrical stimulation of the right median nerve was performed at the wrist through a bipolar electrode (cathode proximal), using constant current square wave pulses (duration, 1 msec) at an intensity of three times the perceptual threshold (Digitimer, Welwyn Garden City, Herts, United Kingdom). All subjects were asked to focus on the stimulated hand during the induction protocol.

The inter-stimulus interval (ISI) between peripheral median nerve stimulation and TMS of the primary motor hand area was set to be equal 25 msec. This interval has been shown to be optimal for inducing a sustained increase in motor cortex excitability (15).

MEP amplitude measurements (average of 20 responses) were performed at baseline (B) and every 15 min for 60 min (P15, P30, P45, P60) after the associative stimulation. Data were expressed as percentage of the baseline.

Rodents' Experiment

Animals. We used pathogen-free, 20–25 g heterozygous double transgenic mice expressing both human APP (K670N:

Table 1. Subjects Demographic and Clinical Characteristics

	AD	Controls
Mean Age, years	70.1 ± 7.4	68.4 ± 6.1
Gender, *n* (% female)	40%	40%
Time Since AD Diagnosis (years)	1.2 ± .67	
Mean MMSE	20.02 ± 3.9	27.9 ± 1.8
Mean ADAS-cog	24.4 ± 8	8.8 ± 3.5
Mean MADRS	5.8 ± 3.7	6.21 ± 3.6

Values are expressed as mean ± SD. AD, Alzheimer's Disease; MMSE, Mini-Mental State Examination; ADAS-cog, cognitive subscale of the Alzheimer's Disease Assessment Scale; MADRS, Montgomery-Asberg Depression Rating Scale.