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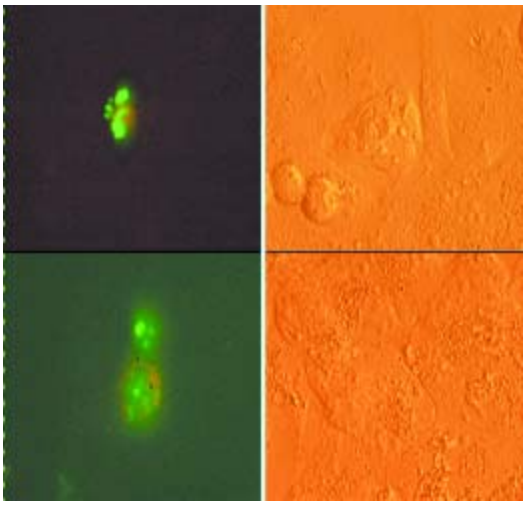

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Reviews

Articles

Scientific board

Towards Harmonisation of Caregiver Outcome Measures

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On behalf of the Medical and Scientific Panel of Alzheimer's Disease International Working Group on Consensus Guidelines for Caregiver Research

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Abstract

Unlike standard measures of cognition or activities of daily living employed in dementia research, outcome measures used in caregiver intervention studies are far from uniform. Because of the variety in the measurement of intervention effectiveness, comparison between studies is difficult except by statistical manipulation. An international group of caregivers and experts in the field of caregiver research met at the World Alzheimer Congress in Washington D.C. in July 2000. Their aim was to seek agreement on issues of importance in caregiver research, and to achieve harmonisation with regard to which caregiver outcomes should be assessed and how these should be measured. Subsequent to the symposium, a questionnaire was sent to the participants to finalise the minimum data set of recommended caregiver outcome measures. The task, far more complex than initially realised, will require further discussions with caregivers and researchers separately and together. This paper presents the basis of a minimum data set for further refinement and discussion.

Keywords: caregiver, carer, harmonisation, outcome, assessment, measure, dementia.

Why do we need harmonisation?

Unlike standard measures of cognition or activities of daily living, which are used to test the effectiveness of compounds in drug trials for people with dementia, outcome measures used in caregiver intervention studies are far from uniform. Interventions for caregivers, such as counselling, training/education programs, support groups and various combinations thereof, have attempted to effect change on a plethora of measures. These include, but are not restricted to, psychological morbidity (e.g. depression, anxiety), physical health, quality of life, social support, burden (objective and subjective), knowledge of Alzheimer's disease (AD), health care utilisation and expenditure, coping styles, relationship strain (between caregiver and patient) and activity restriction. The effects on variables pertaining to the person with dementia,

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such as cognition, function, problem behaviours, depression, institutionalisation and death, have also been considered. Given the wide variety of indicators of effectiveness employed, comparison between studies is difficult except by statistical manipulation.

Harmonisation of caregiver intervention outcome measures is desirable for a number of reasons [1]. Firstly, it would result in a greater emphasis on observable, measurable factors in intervention studies. This in turn would enable the exploration of interactive (e.g., content and delivery method) and additive effects of interventions. Finally, it would also facilitate the execution of meta-analyses and optimisation analyses, by making it possible for data from multiple studies to be pooled.

Method

With harmonisation in mind, a group of caregivers and experts in the field of caregiver research, representing different cultures and countries from Africa, Asia, Australia, Canada, Europe, North America, South America, South Africa and the United Kingdom, met at the World Alzheimer Congress in Washington D.C. in July 2000. Their aim was to seek agreement on issues of importance in caregiver research and to achieve agreement as regards to which caregiver outcomes should be assessed and how these should be measured. The concept of harmonisation, as defined by the International Committee on Harmonisation, implies "a group process working toward a mutually satisfying end, not a process wherein some were made to submit to rigid standards imposed by others" [2]. This meeting utilised a 'top down' (from experts) and 'bottom up' (from caregivers) approach in order to reach consensus on important outcome measures in caregiver research. General issues pertinent to the field were discussed, the main points extracted for the purpose of guiding the choice of a minimum data set of essential outcome measures, and issues clarified by subsequent postal questionnaire. This paper aims to summarise the discussion and ensuing recommendations and present issues pertinent to caregiver evaluation research and ultimately achieving harmonisation of caregiver outcome measures.

General issues that emerged regarding outcome measurement

Situation-specific factors

Interventions cannot be viewed as 'one size fits all'. The effectiveness of a particular intervention may be influenced by variables pertaining to the caregiver, the person with dementia and the study itself, such as the relationship between caregiver and person with dementia (spouse, adult-child, other – different roles and responsibilities), stage of dementia (different problems posed at early, middle, or late stage), context of the interventions (home, day care, nursing home), length of the intervention and the duration of follow-up (as there may be a latency before benefits become apparent and some effects may only be transitory). Caregivers may be providing care for more than one person (e.g. for a spouse and parent); this needs to be taken into account when measuring outcomes.

Choosing realistic outcomes

It is important to be realistic about what can and cannot be changed when selecting outcomes. Some outcomes represent the accumulation of a lifetime of exposures, habits or personality styles and are therefore very difficult to change in a brief intervention (e.g. chronic illness, self-esteem). Therefore, when selecting outcomes, it is important to be aware of how close (proximal) or far (distal) the primary outcome is to or from the goal of the intervention. For example, teaching caregivers how to cope with behavioural problems may reduce caregivers' distress in response to aggression (a proximal measure) but is less likely to influence caregivers' feelings of self-worth (a distal measure). A related issue is the need to match sufficiently specific and sensitive outcome measures to the expected effects of an intervention. While change in one domain (such as improved coping skills) may spill over into other domains (such as less depression), it is better to specify the most likely effects of treatment and to choose outcome measures accordingly.

Cross-cultural issues

Harmonisation of outcome measures would enable valid comparisons between regions, studies and cultures. One of the difficulties with cross-cultural research is the lack of clarity about whether observed differences are genuine or reflect cultural bias in what one is trying to measure (e.g. due to assumptions of literacy). Care is necessary lest the richness of caregiving in other cultures is lost by forcing its assessment into Western conceptualisations, for example by ignoring spirituality in quality of life measures. The concept of burden varies across cultures; in some regions, such as India, cultural norms may prevent caregivers from admitting to strain.

Caregiver-led research

In order to direct the course of future research, one of the aims of this symposium was to discover which outcome measures were important to caregivers. Contrary to the expectations of researchers, a reduction of psychological effects of caregiving such as depression or subjective burden were not mentioned by caregivers as important, who gave greater weight to practical, information and

financial outcomes. Caregivers valued interventions that provided practical assistance to them, and those that led to an improvement in the quality of life of both the caregiver and the person with dementia. Surprisingly, delay of institutionalisation (or nursing home placement) of the person with dementia was not discussed, although often interventions that delay institutionalisation are extolled as positive outcomes[3,4]. Attitudes toward institutionalisation vary; keeping people at home is not necessarily the best outcome. A delay in institutionalisation might be a negative outcome if people on waiting lists want to be in care and do not want to be a burden. Perhaps the resolution should be to aim for caregiver-led research. In order to achieve harmonisation, we need to survey the experience of a wide variety of people and to canvass many diverse views. An allied approach is Goal Attainment Scales[5], where outcomes are tailored to the individual.

Dissemination of research findings to caregivers and clinicians

Caregivers identified as a priority the bridging of the gap between research and practice, between academics and clinicians, and between health care professionals and people with dementia and their caregivers. Similar arguments apply to information about services and resources. Caregivers stressed the importance of information as a means to empowerment, and expressed concern that without knowledge "sometimes out of the goodness of your own heart you may be doing the wrong thing". Caregiver knowledge emerged as an important research outcome.

Recommended minimum data set of outcome measures for caregiver interventions

Basic information

Participants were in agreement that certain basic data should be reported with regard to the characteristics of the intervention used (description of content, number and duration of sessions, time span, size of groups, length of follow-up), the person with dementia (age, sex, marital status, ethnicity, years of education, main occupation, living arrangements, diagnosis criteria and source, severity of cognitive and functional impairment), and the caregiver (age, sex, marital status, ethnicity, years of education, main occupation, relationship to person with dementia, other caregiving responsibilities. Situation-specific information must be taken into account when assessing outcomes.

Specific outcome measures

A model of caregiver burden [6,7] provides a basis for identifying suitable outcomes to measure and facilitates the choice of appropriate proximal measures. This model shows that caregiver strain may fall into four categories – psychological, physical, financial and social – each with distinct outcome measures. In addition, there are a number of protective and exacerbating factors that can be identified and measured. References to and details of specific measures are provided in the text or the accompanying tables but not in both.

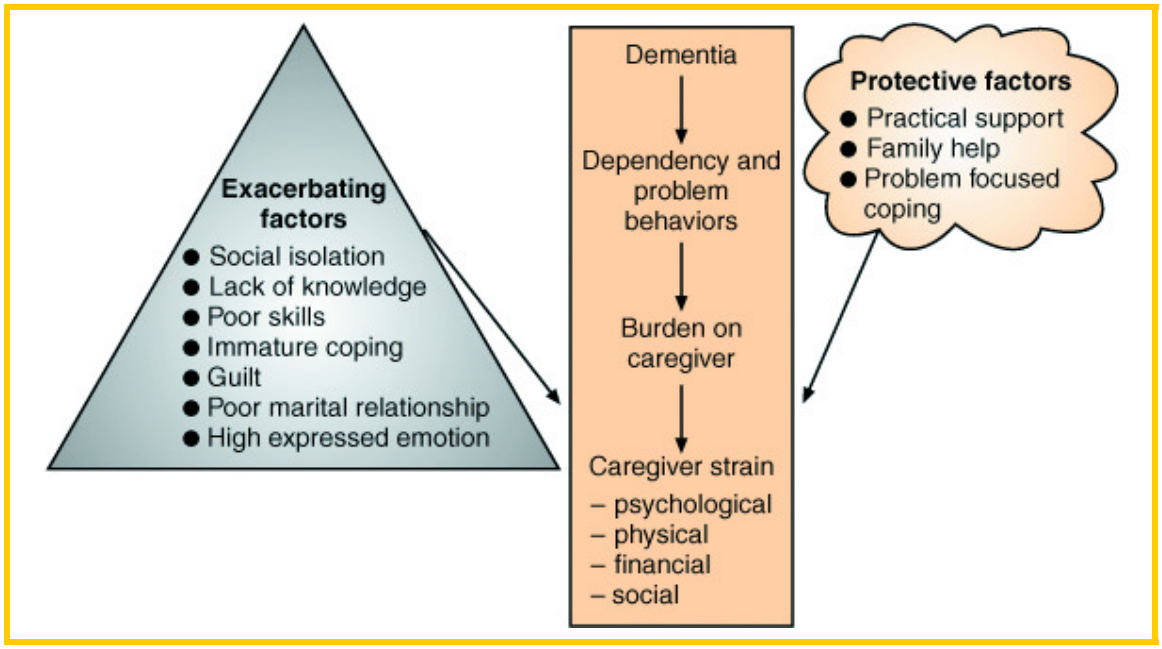

Figure 1. Model of Caregiver Burden (after Poulshock & Deimling, 1984)

Psychological

The characteristics, reliability and validity of a selection of caregiver outcome instruments that measure burden and strain, quality of life, depression and other psychological morbidity, and their ability to address the general issues discussed above, are tabulated (Table 1).

Physical

Caregivers' physical health may be adversely affected by the strain of caregiving. These effects may manifest in a worsening of chronic conditions e.g. hypertension, greater usage of medical services and increased consumption of prescription medication. Number of visits to doctors and volume of medication consumption can be readily

Table 1: Recommended measures of psychological outcomes

Burden and Strain

Instrument	Description	Reliability	Validity	Comments
Burden Interview (BI) [19,20] USA	A 22 item inventory that measures the degree to which caregivers perceive their caregiving responsibilities as having an adverse effect on their health, personal and social life, psychological well-being, and finances. Each item is rated on a 5-point scale (ranging from 'never' to 'nearly always present'). Total scores range from 0-88, with higher scores signifying higher levels of burden.	Internal reliability: Alpha of 0.91 [21]. A Japanese version of the BI had α = 0.93, & the single global rating of burden (Q22) on the BI and the sum of other BI items were significantly correlated (r = 0.65) [22] Test re-test reliability is 0.71 [21]	Construct validity: burden was negatively related to morale and positively related to hours spent giving care[23]. CES-D and BI total were significantly correlated (r = 0.67).	This scale has been adapted for use in other cultures, e.g. Japan.
Screen for Caregiver Burden (SCB) [24] USA	A 25-item questionnaire designed to measure objective and subjective burden among spousal caregivers. Items assess various domains, including patient behaviours, disruptions in family and social life, and caregiver affective responses. SCB yields two scores, objective burden (prevalence of caregiving experiences) and subjective burden (ratings from 1-4 of distress in relation of each experience). Subjective burden scores in excess of 42 considered 'quite high'.	Internal reliability coefficients are 0.85 (OB) and 0.88 (SB).	Construct validity was supported by relationships of patient behavioural and cognitive functioning with OB and caregiver distress and personality variables with SB. Criterion validity (differences in burden between AD caregivers versus controls) was demonstrated by using age- and sex-matched controls. There were also relationships between increases in SB/OB and deterioration of patient functional/behavioural functioning and SB/OB increases in caregiver distress.	Sensitivity to change was examined by using a sample of individuals with early- to mid-stage AD: over 15-18 months a majority of caregivers changed by a value that was greater than would be expected by measurement error alone. This scale has been used successfully in Germany.

Quality of Life

Instrument	Description	Reliability	Validity	Comments
Satisfaction with Life Scale (SWLS)[25] USA	5-item questionnaire that measures global life satisfaction. Caregivers indicated their agreement with each of the 5 questions on a scale from 1 (strongly disagree) to 7 (strongly agree). The possible range of scores is 5 (low satisfaction) to 35 (high satisfaction).	The test-retest correlation coefficient was 0.82, and coefficient alpha was 0.87. Internal consistency was examined by calculating item-total correlations for the 5 items were 0.81, 0.63, 0.61, 0.75 and 0.66.	The correlation between the SWLS and the Cantril Self-Anchoring Striving Scale was found to be 0.62 in college students and 0.66 in an elderly sample.	Has been used in a variety of populations e.g. Iranians living in Norway, Arab Israelis, and Nepalese.

Table 1: Recommended measures of psychological outcomes *(continued from previous page)*

Depression

Instrument	Description	Reliability	Validity	Comments
Centre for Epidemiologic Studies Depression Scale (CES-D) [26] USA	20-item self-report of depressive symptoms rated according to frequency of occurrence, from 1 (rarely) to '5' (most of the time). Scores range from 0-60, with higher scores indicating more depressive symptoms. A score of 16 is the cut-off for designating subjects likely to be experiencing a significant level of depression.	Has been shown to have a high internal consistency, with coefficient alphas of 0.90 for psychiatric patients and 0.85 for non-psychiatric individuals		This is a widely used scale. Self-report structure is useful for research purposes. It has been used in a variety of cultural settings – Greece, Spain, Hong Kong, and in Puerto Rican- and African-Americans in USA.
Beck Depression Inventory (BDI) [27] USA	Self-report questionnaire of depressive symptoms (inc. mood, vegetative, and cognitive aspects of depression) presented in multiple-choice format. The caregiver chooses between four-statements that most appropriate to how they have been feeling over the past week. The maximum score is 63. Scores of 0-9 indicate the normal range, 10-15 indicate minimal depression, 16-19 indicate mild-moderate depression, 20-29 indicate moderate-severe depression, and 30-63 indicate severe depression.	Test-retest reliability with 38 patients was above 0.90. Spearman-Brown reliability was 0.93 and internal consistency for test items 0.86 [28].	Concurrent validity coefficient with the Zung SDS is 0.79 in psychiatric patients and 0.54 in college students [29]; with the Hamilton Rating Scale is 0.82 in psychiatric patients.	This is a well-known and very widely-used scale. Self-report structure is useful for research purposes. It has been used in countries such as Finland, Saudi Arabia, Malaysia and Taiwan.
Hamilton Rating Scale for Depression (HRSD) [30] UK	17-item interviewer checklist with total scores ranging from 0-52, representative of the severity of depression. Scores higher than 14 indicate the presence of depressive symptomatology.	Inter-rater reliability = 0.90. 15 of the individual items had reliability scores which ranged from fair (0.40 to 0.59) to excellent (0.75 –1.00), items which fared poorly included paranoid symptoms, depersonalisation/derealisation, insight, obsessive/compulsive symptoms, hypochondriasis and guilt.[31]	Factors: retarded depression, mixed and somatic symptoms and anxiety reaction.	While this scale is very widely used (in countries such as Korea, Japan, Italy, Austria, Russia and Spain), it is clinician-administered and thus not as amenable for research purposes as other depression scales.

(to be continued in the next page)

measured and used as indices of physical health. Caregiver health may also be measured quantitatively by using omnibus measures of physical health (and well-being), such as the OARS [8] or the widely-used SF-36 [9], self-rated health scales, or more specifically by examining individual functions such as immune functioning [10,11]. A different approach is to examine caregivers' self-perceptions of health, using instruments such as the Subjective Overall Physical Health Questionnaire [12], a brief scale which asks caregivers to rate their own health, and their perception of their health in comparison to others of the same age. The internal reliability of this scale is high ($\alpha = 0.85$).

Financial

Caregiving may result in reduced income and /or increased costs for the caregiver. The following are taken into account when assessing financial burden:

- Loss of earnings by the patient or the caregiver
- Increased expenditure on medical consultations and investigations, drugs, personal care and nursing care
- Costs associated with respite or residential care
- Increased time spent caregiving
- Welfare payments

Table 1: Recommended measures of psychological outcomes *(continued from previous page)*

Other Psychological Morbidity

Instrument	Description	Reliability	Validity	Comments
General Health Questionnaire (GHQ) [32] UK	Self-administered screening instrument to aid in the detection of non-psychotic psychiatric illness. There are several versions of the GHQ. The original scale consists of 60 items, but there are also 36-, 30-, 20-, and 12-item versions. On the GHQ-30, where scores range from 0-30, scores > 4 are taken to indicate cases of probable psychiatric illness. Items include questions about psychological distress and altered behaviour. For each item, caregivers are asked to compare their recent state with their usual state. Items are rated on a 4-point scale where the essence of the ratings are 'not at all', 'same as usual', 'rather more than usual', or much more than usual'. An item is counted as being present if it is experienced 'rather' or 'much' 'more than usual'.	The test-retest reliability for the 60-item is 0.76, for the 30 items is 0.77, for the 20-item is 0.73, and for the 12-item is 0.73.	Construct validity has been examined in both a GP setting and in a medical outpatient population. The coefficients for the GP population were 0.80 (60 item), 0.80 (30-item), 0.77 (20 item) and 0.77 (12 item). The coefficients for the outpatient population were 0.77 (60 item), and 0.72 (30-, 20- and 12- item). Sensitivity in the GP population was 95.7% (60 item), 91.4% (30 item), 88.2% (20 item) and 93.5% (12 items). Sensitivity in the outpatient population was 80.6% (60 item), 64.5% (30 item & 20 item) and 74.2 % (12 item). Specificity for the GP population was 87.8% (60 item), 87% (30 item), 86% (20 item) and 78.5% (12 item). Specificity for the outpatient population was 93.3% (60 item), 91.6% (30 item), 96.7% (20 item) and 95% (12 item).	The GHQ is widely used in the rating of psychological distress. The shorter versions of the GHQ are commonly used for research purposes. It has been used in Japanese, Nigerian, Polish, Afghani and Greenland Inuit samples. Alternative scoring methods have been reported. The GHQ can be scored by scoring as positive the 'same as usual' responses on the 15 negative items (chronic GHQ method: CGHQ), which is considered more appropriate for chronically stressed caregivers [33].
Positive and Negative Affect Scale (PANAS) [34] USA	Contains ten indicators of positive affect (PA) (e.g. excited, enthusiastic, inspired) and 10 of negative affect (NA) (e.g. distressed, nervous, scared). Respondents rate each of the indicators on a scale that ranges from "1" (very slightly or not at all) to "5" (extremely). A higher score suggests a greater degree of positive or negative affect. Ratings can be gained with several different temporal instructions: how they felt (a) 'right now (that is, at the present moment)' (moment instructions); (b) 'today' (today); (c) 'during the past few days' (past few days); (d) 'during the past week' (week); (e) 'during the past few weeks' (past few weeks); (f) 'during the past year' (year); and (g) 'in general, that is, on the average (general).	Internal reliability of the PANAS ranges (over the different time periods) from Cronbach's coefficient α 0.86 (year) to 0.90 (today) for the PA and from 0.84 (year) to 0.87 (today, general) for the NA. Test-retest reliability ranged from 0.47 (today, past week) to 0.68 (general) for the PA and from 0.39 (today) to 0.71 (general) for the NA scale.	Convergent validity: Correlations between the PANAS and other brief affect measures range from 0.76 to 0.92. Correlation between the Hopkins Symptom Checklist (HSCL) which measures general distress and dysfunction ranges from 0.65 (today) to 0.74 (past few weeks) with the NA. The correlation between the Beck Depression Inventory and the NA ranges from 0.56 (past few days) to 0.58 (past few weeks). Discriminant validity: the correlation between the NA and the PA scales ranged from -0.12 (today) to -0.23 (year) over the different time periods. Correlation between the Hopkins Symptom Checklist (HSCL), which measures general distress and dysfunction ranges from -0.19 (past few weeks) to -0.29 (today) with the PA. The correlation between the Beck Depression Inventory and the PA ranges from -0.35 (past few days) to -0.36 (past few weeks).	This is a flexible scale and is broad-ranging enough to be useful in a range of research situations. It has been used in the Netherlands, Norway and South Africa.

Table 2: Recommended measures of social support and isolation

Instrument	Description	Reliability	Validity	Comment
Instrumental and Expressive Social Support Scale (IESS)[35] USA	The IESS is 28-item scale that measures the perceived adequacy of available support from the social network, as a function of disruptions in relationships. Items are a list of problems covering finances, time and effort demands, lack of adequate companionship, communication, dependency, familial and household problems. Respondents are asked how they have been bothered by each item over the past 6 months and respond on a scale of 1 (most or all the time) to 5 (never), and item scores are summed. Possible scores range from 22 –130.	Internal consistency: a ranged from 0.89 to 0.93 over two time periods. Confirmatory factor analysis and reliability tests show that excess of responsibility/demands, lack of money and unsatisfactory intimate relations factors show strong internal consistency and stability over time	Five factors: excess of responsibilities/demands, lack of money, lack of involvement, unsatisfactory intimate relations and family problems. Scores on the IESS strongly correlates with depression measured with the CES-D [26]. But regression analyses show that factors are conceptually different from depression.	
Norbeck's Social Support Questionnaire (NSSQ)[36] USA	Self-administered measure designed to measure multiple dimensions of social support. The NSSQ measures 3 functional properties of social support (affect, affirmation and aid) and two network properties (duration of relationships and frequency of contacts). The subject is asked to list up to 20 significant people in their life, and to specify the relationship of each person. Subjects then rate each person on a 5-point scale (for questions 1 to 6 and 9b, 1 (not at all) to 5 (a great deal); for question 7, 1 (less than 6 months) to 5 (more than 5 years); for question 8, 1 (once a year or less) to 5 (daily) for 9 questions. The sum of ratings for a question gives the respondents score for that question except for question 9. Question 9, losses is scored as a yes, no response, the quantity of losses is the number of categories in which the subject has loss an important relationship and the quality of losses is the rating on the 5-point scale.	Test-retest reliability ranged from 0.85 to 0.92, All items except Q9 are significantly inter-correlated, range from 0.69 to 0.98.	Significant correlations between some items and related subscales on the Social Support Questionnaire [37]. Lack of significant relationships with the Profile of Mood States.	This scale has been used in African-American, Hispanic, Filipino, Mexican and Arab samples in the USA.
Stokes Social Network Scale [38] USA	Caregivers construct a Social Network List, by listing "the initials of up to 20 people who are significant in your life and with whom you have contact at least once a month". Relatives form their Social Network, and people they can "confide in or turn to for help in an emergency" form their network of friends. The Satisfaction with Social Network Scale is an eight-item scale that asks caregivers to rate both their social networks (as defined by the Social Network List) and their networks of friends (people from the Social Network List who are not relatives) on four dimensions: general satisfaction with the network, amount of desired change in the network, satisfaction with assistance in daily activities, and satisfaction with emotional support. Each of these dimensions is rated on a scale from 1 (very satisfied) to 6 (very dissatisfied).	Internal consistency: $\alpha = .92$		
Social support questionnaire[39] USA	Social support was measured in three ways: the amount of interaction with people in the caregivers' informal support network, the amount of assistance provided by others, and the caregivers' rating of the adequacy of social support. Interaction with one's support network was determined by asking subjects to identify relatives and friends who were important to them, and then assessing the frequency of phone contact and visits with these persons. Assistance with caregiving tasks (e.g. day care, respite care) was assessed by summing the amount of help from both informal and formal services. The caregivers' subjective rating of the adequacy of the support was measured using a 4-point scale, ranging from 4 (having all the help needed) to 1 (feeling without support and assistance			This scale has been used in Japan and Hong Kong.

Social

The demands of caregiving may lead to the abandonment by the caregiver of hobbies and social activities, in addition to the social interaction lost by giving up work. Level of social support and perceived isolation may be measured by a number of instruments (Table 2).

Measurement of social support is fraught with complexity. Actual support received may differ considerably from perceived support, the rating of which may be highly influenced by the respondent's psychological state. For some caregivers, practical support is more important than emotional support, for others the reverse holds. The effects of formal support (by paid or professional staff) differ from those of informal supports, i.e. friends and family. Further, simply counting number of visits to the home, e.g. by family members, is too crude as such visits may be a source of distress or of succour. Male and female caregivers, spousal and child caregivers use supports differently. Finally, correlations between support levels and outcomes are difficult to interpret. If one finds that more distressed caregivers receive more support this may be interpreted as a lack of efficacy or as demonstrating that they have a greater need for help [13,14].

Exacerbating factors

Factors identified as having the potential to exacerbate existing burden include poor caregiver physical health and social isolation, which have been addressed above, caregivers' level of knowledge, their coping skills and the premorbid relationship between caregiver and care recipient. Knowledge can be measured quantitatively, for example by the Alzheimer's Disease Knowledge Test (ADKT) [15]. This comprises twenty multiple-choice items concerning facts about AD. This scale has high internal consistency (α = 0.71 to 0.92) and moderate test-retest reliability (α = 0.62). It has been shown to be sensitive to changes in knowledge level after educational interventions. However knowledge has advanced since this was devised and there is a need for an updated scale. A number of instruments have been designed to measure coping style and skills and premorbid relationship, particularly premorbid marital relations (see Tables 3 and 4).

Problem behaviours in the person with dementia, such as aggression, shadowing and constant questioning, and psychiatric symptoms such as depression, delusions and hallucinations are a major cause of caregiver burden [16]. Both the behaviours themselves and the caregivers' reactions to them are important to measure. If no behaviours occur there can be no reactive distress. If after an intervention for troublesome behaviours caregiver

Table 3: Recommended measures of coping

Instrument	Description	Reliability	Validity	Comments
Health Specific Family Coping Index for Non-Institutional Care (HSFCI) [40] USA	Measures the caregivers' coping skills, by rating potential and actual health problems in the psychosocial and physical domains of health. Coping is rated in 9 domains, each with scores ranging from 1-5. Domains include Physical Independence, Therapeutic Competence, Knowledge of Condition, General Hygiene, Health Attitudes, Emotional Competence, Family Living, Physical Environment, and Community Resources. A summary score is obtained by summing sub-scores in each domain (min = 9, max = 45)	Inter-rater reliability: α = 0.99	Construct validity: correlation between HSFCI and General Aptitude Family Coping Index is 0.94	
Family Crisis Oriented Personal Evaluation Scale[41] USA	30-item scale that identifies the problem-solving and behavioural strategies utilized in difficult situations. Five subscales: acquiring social support, reframing, seeking spiritual support, mobilising the family to accept/acquire help, and passive appraisal.	Internal reliability has been established at 0.77, with test-retest reliability at 0.71.	Construct validity: determined using a factor analysis procedure.	This scale has been used in the USA and in Austria.
Dementia Management Strategies Scale (DMSS) [42] USA	Caregivers rate how often during the past month they have used 28 dementia management strategies, on a scale from 1 (never) to 5 (most of the time). Summary scores are generated for three subscales: Use of Criticism, Use of Encouragement, and Active Management.	Reliability of the three subscales ranges from 0.77 to 0.85.		
Decision-making confidence and skill [43] USA	Caregivers respond to 14 items on a scale ranging from 1 (strongly agree) to 5 (strongly disagree). Responses are summed, with possible scores ranging from 14-70. Higher scores indicate more confidence. Caregivers are then asked to think of a recent important decision and the alternatives they considered. Higher scores indicate better decision- making skill.	Cronbach's α has been found to be 0.86 [44].		

Table 4: Recommended measures of caregiver relationships

Instrument	Description	Reliability	Validity	Comments
Marital Communication Scale [45] USA	This 10-item scale taps the caregivers' feelings, beliefs, and attitudes regarding communications with their spouses/partners. Ratings range from 1 (strongly disagree) to 5 strongly agree).	Internal reliability was 0.68, with test-retest reliability being 0.90.		
Family Satisfaction Scale [46] USA	This 14-item scale measures family members' level of family satisfaction on two dimensions: cohesion and adaptability.	Internal reliability for the scale was calculated at 0.92, with test-retest reliability of 0.75 at 5 weeks.	Construct validity was established using factor analysis procedures.	
Family Support Scale – modified [47] USA	7-item scale, which measures the caregivers' perception of family assistance in caring for the patient. Ratings range from 1 ('none of the time') to 3 ('most of the time').	Internal reliability: Cronbach's alpha = 0.82.		
Negative impact on elderly-caregiver family relationship (ECR) [7] USA	An 11-item, factor-derived scale, which assesses family members' negative affect toward a relative, including feelings of anger and resentment.		Has been found to be related to disruptive behaviour of the patient, and of the patient's impairment in activities of daily living.	
Marital Needs Satisfaction Scale [48] USA	Consists of 24 items designed to measure the extent of marital need satisfaction of older persons. Scores range from 0 to 225 with a higher score indicating greater satisfaction of marital needs. The six dimensions of spousal satisfaction that were assessed included love, respect, communication, personality fulfilment, life meaningfulness, and integration of life experience. Subjects respond on a 5-point scale (very satisfactory to very unsatisfactory).	Internal consistency was measured using the chi-square test and each item was found to be discriminating between subjects scoring in the highest and lowest quartiles at the 0.001 level of significance. Split-half reliability is 0.99. $\alpha = 0.97$ [49].		

distress subsides, this may be because the behaviours have abated or because the caregiver is reacting with more equanimity to the same behaviours. Two widely-used instruments that measure caregivers' reactions to such behaviours are the Revised Memory and Behaviour Problems Checklist (RMBPCL) [17] and the Neuropsychiatric Interview (NPI) [18]. The RMBPCL is a 24-item checklist completed by the caregiver that yields a total score as well as three sub-scores: memory-related, depression, and disruptive behaviours. The NPI comprises 12 behaviours and psychiatric symptoms rated by a clinician after interviewing a caregiver with regard to frequency, severity and caregiver's distress in reaction.

Protective factors

Factors identified as protective against caregiver burden include practical support (Table 2), and adaptive coping mechanisms (Table 3).

Conclusion

We report here on the outcome of an international discourse on harmonisation of caregiver outcome measures. This paper presents a basis for this discourse and for refinement.

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The Role of Cognitive Stimulation in Diagnosing Mild-Cognitive-Impairment Subjects at Risk for Alzheimer-Type Dementia

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Abstract

Some elderly individuals exhibit significant memory deficits but do not have dementia. Their general intellect is preserved and they have no impairment in everyday activities. This condition known as Mild Cognitive Impairment (MCI) may be considered as a pre-clinical stage of Alzheimer's disease, but not all MCI patients develop dementia. Stimulating cognitive reserve capacity may help identify those who will progress to Alzheimer's and those who will remain stable.

Keywords: MCI, cognitive stimulation

Introduction

There currently exists an abundance of studies and published research concerning the diagnosis and treatment of people suffering from dementia. Regarding Mild Cognitive Impairment (MCI), the number of epidemiological trials continues to grow, but no consensus has been reached as to the precise definition of MCI, nor consequently, to its diagnostic criteria. As far as diagnostic and treatment of MCI patients is concerned, a great number of possibilities remain open for exploration. In this study we discuss the role of the mobilization of cognitive reserve capacity in diagnosing MCI subjects at risk for Alzheimer-type dementia.

A means of therapeutic intervention: Cognitive Stimulation (CS)

Cognitive Stimulation (CS) is a therapeutic non-pharmacological treatment that aims to optimize cognitive function based on the notion of cerebral plasticity. This method is concerned with memory loss resulting not only from dementia but also from the normal aging process. For patients who complain of memory loss while demonstrating normal cognitive ability, CS offers a program that aims to dedramatize their mnemonic difficulties and to facilitate the return of self-confidence through the acquisition or re-learning of strategies for memorisation. Even in the absence of objective cognitive impairment, all complaints of mnemonic difficulties should be taken into consideration, as they may be a sign of future deterioration. It would seem appropriate that all complaints concerning mnemonic difficulties to be taken seriously and responded to appropriately, even in the absence of an identifiable pathology.

As to Alzheimer's disease, it is hypothesised that these patients present a certain reserve capacity for cognitive information processing, and that while this capacity is certainly limited, it can still be stimulated. This reserve capacity should allow the patients to benefit in a small measure from cueing and mnemonic strategies. In Alzheimer's patients, implicit memory which is preserved for a longer period than episodic memory, also responds to regular stimulation. Offering patients a set of exercises that takes this remaining reserve capacity into account allows them to maintain, for a certain period, a level of cognitive performance superior to that of un-stimulated subjects.

The methods used in CS are designed to maintain, as long as possible, patients' daily functioning. The exercises are intended to stimulate the various areas of cognition: memory, concentration, language, the executive function,

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spatio-temporal orientation and visuoconstructive abilities. The patients apply strategies based on mental imagery, categorical classification, and semantic association, with the aim of preserving and even improving episodic and semantic memory as well as consolidating implicit memory.

The stimulation exercises are developed in relation to the principle interests and activities of the elderly patient. They are grouped by theme. Each theme contains exercises of different types, focusing on memory, concentration, linguistic, and executive abilities. CS thus offers an approach that incorporates all the different aspects of cognitive activity. Based on the notion that one's psycho-affective state and cognitive functions are interdependent, this global vision of therapy permits treatment to be extended to the psycho-social functioning of the patient. Motivation in particular plays an important role in the intellectual function. In turn, the cognitive and psycho-affective functions influence the social role of the patient, namely his or her involvement in family activities, maintenance of social relationships, and participation in social activities. The strict framework of the CS sessions (organized in groups of 8 to 10 people and led by a psychologist) permits the patient to meet others with similar difficulties, which in turn serves to reduce anxiety with respect to one's individual situation.

CS as a means of identifying "at risk" subjects

With respect to patients suffering from MCI, CS has an other objective: it seeks to identify the MCI subjects at risk, those whose condition could potentially develop into Alzheimer's disease. It is presumed that CS-intervention may slow down the rate of overall cognitive decline and possibly delay onset of disabling symptoms in MCI subjects. The reserve capacity and the considerable plasticity of the human brain accounts for a large degree of variability in the performances in cognitive activities evidenced by stimulation exercises. In particular, traditional encoding and retrieval strategies require intact learning resources thus a minimum of reserve capacity. Consequently, non-responsiveness to CS showed by impaired cognitive processing patterns on stimulation exercises refers to reduced cognitive reserve capacity and may contribute to early identification of incipient dementia.

CS attempts to distinguish the amnestic MCIs from the"stable" MCIs. The "stable" MCIs are those who display, at a given moment in time, mnemonic or cognitive performances that fall below the norm (taking into account their age and socio-educational level) but who are not expected to develop dementia. These subjects may even improve with time. SC offers these patients a program that permits them to learn cognitive strategies, which, in turn, permit them to improve their cognitive functions.

In this regard, two new questions on the state of pre-clinical dementia research are currently at the fore:

- Do all subjects who present with MCI necessarily develop Alzheimer's disease?
- What is the precise nature of the mnemonic and cognitive functions in the pre-clinical phase of Alzheimer's disease?

Mild Cognitive Impairment designates a clinical state whereby the subject presents sub-normal mnemonic and/or cognitive abilities that have no impact on his or her daily activities and does not reveal the presence of dementia. As far as aetiology and prognosis are concerned, the population affected by MCI is heterogeneous. Even if subjects with MCI are considered to be an "at risk" population, we know that not all MCIs develop into dementia. The most frequently quoted statistics are those issued by the Mayo Clinic: 15-20% per year [1].

There is a theoretical divergence concerning the origins of MCI. In cases where Alzheimer's disease begins with memory loss, the hypothesis is that the pre-clinical stage of the disease corresponds to that of a subject with amnestic MCI. This hypothesis is supported by the notion that brain deterioration begins well before the clinical characteristics of Alzheimer's disease manifest themselves. The hippocampus and other structures near the temporal medial lobe known to be important for memory function (such as the parahippocampal gyrus, the entorhinal cortex), are the initial sites of neurodegeneration in Alzheimer's disease. For others, the onset of Alzheimer's may be signalled by problems other than memory loss, such as difficulty in the executive functions.

This MCI-multiple cognitive domains deficits-type consists in a profile associated with below average-for-age cognitive performances other than memory, that do not reach the level of dementia. Individuals show a greater rate of decline in selected cognitive abilities than normal elderly. These MCI subjects show mostly frontal lobe function impairment: problem solving deficits, abstract thinking deficits, loss of mental flexibility, response inhibition, attentional deficits, and visuospatial deficits.

Currently, we do not know whether these two subtypes (amnestic and multiple cognitive domains-MCI) correspond to two different conditions of MCI, or if they correspond to two different levels of one MCI-condition. It is most likely, but not certain, that the multiple cognitive domains-subtype correspond to a later stage of amnestic MCI, when the underlying histopathology has progressed from entorhinal cortex to hippocampus, on to neocortical association areas and the prefrontal lobe. In that case, all non reversible MCI subtypes might be considered as the preclinical sage of AD. Consequently, CS programmes for MCI individuals have to focus on memory- as well as on langage- and executive function stimulation exercises.

However, clear impairment in at least one cognitive domain in addition to memory decline is helpful in

identifying individuals that are going to convert to Alzheimer's as the risk of dementia is significantly increased among subjects with clear cognitive impairment beyond memory loss[2]. Predictive accuracy is extremely high for individuals who have simultaneously a memory complaint, global cognitive impairment and exhibit episodic mnemonic deficits.

Neuropsychological screening tests play a key role in clarifying the diagnostic question. However, these tests must be sensitive enough to detect the first signs of cognitive deterioration. Although we do have efficient tests for the detection of early memory loss -Grober & Buschke[3], The 5-word Test[4], the Profile of Cognitive Efficiency [5]- we do not currently have tests capable of detecting slight changes in the executive function.

Neuropsychological tests only reflect the performance of the patient's cognitive function at a given moment in time; they do not provide information on long-term evolution. Follow-up sessions with patients are essential in determining which subjects are likely to develop dementia and those whose performance is likely to improve. A performance that falls below the given norm at a particular moment might well be explained by factors other than latent dementia.

In this context, the CS program developed for MCI subjects may contribute to the detection and prediction of potential Alzheimer's patients. The benefits of CS are the result of both the overall framework of the program and the specific exercises contained therein.

The framework consists of weekly sessions with a psychologist who observes the evolution of the patient's performances, not only in terms of his or her results but, more importantly, in terms of the cognitive procedures that s/he applies during the session. The manner in which the subject processes, learns, and recalls information can reveal the existence of an underlying pathology.

The 12-week sessions also allow the psychologist to detect functional and behavioural changes. Verhey's retrospective study [6] on the psycho-affective behaviour of "at risk" MCI patients yielded the following results: the subjects in the study group (subjects with MCI) tend to visit their general practitioner more often than those in the control group (non-MCI); the MCI subjects tend to suffer more frequently from (mild) depression than the control group; the study group subjects are significantly more affectively dependant on their entourage than the control group subjects; and the MCI subjects are significantly more vulnerable to stress. Verhey refers to this state as the "emotional vulnerability syndrome". This psycho-affective state can be measured by questionnaires that are filled out at the beginning and end of the CS program and by observing the patient's behaviour.

The specific exercises that make up the program are focused on 4 cognitive characteristics commonly considered to be signs of pre-clinical Alzheimer's in amnestic-MCI patients. The 4 MCI cognitive markers are:

- diminished delayed recall,
- diminished category verbal fluency;
- diminished logical memory (paragraph recall),
- diminished associative memory.

Diminished delayed recall:

A meta-analysis of 49 studies on the evolution from MCI to Alzheimer's disease was undertaken by Bäckman [7]. According to this research, the most notable criterion is free-recall in episodic memory. This is consistent with the neuropathologial condition of amnestic MCI: hippocampus and entorhinal cortical areas that are in charge of delayed recall show histopathological deterioration, whereas prefrontal lobe areas, correlated with memory span and working memory interfering with immediate recall, are still preserved [8]. Research data indicate that phosphorylated tau pathology in the ventromedial temporal lobe develops prior to the onset of clinical dementia [9]. The presence of phosphorylated tau pathology is associated with cognitive impairment, particularly impairment of episodic memory. This is evidenced by impaired delayed recall performance and logical memory decline on cognitive screening tests.

Bäckman evidenced that the performance of MCI patients on free-recall tend to remain stable for approximately 3 years due to a reserve capacity that allowed the patient to make use of compensatory strategies. Beyond this period, performances tended to plummet. Bäckman's analysis also demonstrates that not all MCI subjects deteriorate. In certain cases the subject's condition is stabilized or even improved.

Allegri [10] confirms such a deficit in free-recall performance by demonstrating that there is a significant overlap between the results of the "at risk" MCI subjects and those of Alzheimer's subjects, and that an equally significant overlap exists between the free-recall results of "stable" MCI subjects and the number of words recalled by normal subjects. These authors also performed a qualitative analysis of free-recall and were able to determine that "at risk" amnestic MCI subjects produce significantly more intrusions (nearly as many as produced by Alzheimer's patients) than those considered to be "stable".

The CS program contains many exercises focused on the re-learning and optimisation of mnemonic strategies that allow for better retrieval of learned information. "Stable" MCI subjects benefit from these exercises by applying these strategies, which, consequently, improves their delayed recall performance. On the other hand, the "at risk" MCI subjects tend to benefit less from these strategies and their application in such cases is less efficient.

Diminished category verbal fluency:

After free-recall, category verbal fluency is considered to be the second criterion of amnestic MCI. A number of studies have highlighted the pertinence of this criterion. Palmer [11] found a predictable positive value of 89% in a population corresponding to an MMS: 24-30 and to a CDR: 0.5.

All MCI subjects (both subtypes) perform poorly on verbal fluency tests. Impaired verbal fluency may be an early discriminative measure for the progression of dementia. It has to be specified that category fluency is based on a retrieval process that is different from letter fluency. Letter fluency is associated with semi-automatic phonetic processing where as category fluency is associated with multimodal association cortices involved in consciously elaborated word retrieval processes (essentially conceptual processing). Category fluency poses greater demands on the integrity of the semantic network then letter fluency, demanding a strong medial-temporal lobe involvement. MCI subjects showing selective deficits in category fluency may be at risk for AD.

Astell [12] questioned whether this diminished fluency was a result of difficulties in accessing the lexical stock or of a loss of semantic stock. The results show that diminished fluency stems from a reduction in the capacity to identify strategies as well as from the difficulty of applying these strategies.

Each CS session thus contains several vocabulary exercises: synonym-antonym exercises designed to enrich the semantic stock as well as the re-learning of strategies for recalling words or concepts. The goal of these exercises is to improve the subject's ability to compensate when s/he cannot recall a word. Should the subject fail to find a word, s/he would be able to locate a synonym or a description, thus diminishing the anxiety of failure. This is particularly important in that the anxiety of forgetting words in the middle of a conversation constitutes a risk factor for social withdrawal. These compensation strategies will tend to be more efficient in "stable" subjects than in those at risk.

Diminished logical memory:

For Ferris [13], a logical memory test becomes pertinent in diagnosing MCIs „at risk" where the patient presents a CDR 0.5. CS pays special attention to text recall through exercises that focus on learning and reconstituting a paragraph or a very short story. In this case, mnemonic strategies such as categorization and the association of mental images play a primordial role. Not only will "stable" MCI subjects tend to apply these strategies more efficiently and demonstrate a capacity to recall far more information than "at risk" subjects, but they will also increase the number of remembered items as a result of their greater mnemonic reserve capacity.

Diminished associative memory:

Blackwell [14] demonstrated that the ability to learn associated words represents a determinant factor in distinguishing the MCI subjects likely to develop dementia from those who will remain stable. Thus, the performance of "at risk" MCI subjects, determined by the patient's ability to benefit from cueing—a factor inherent in the word association exercises, resembles more closely the performance of early-stage Alzheimer's patients than that of normal subjects. The degree to which the subject is capable of benefiting from cueing is a good barometer for evaluating the amount of reserve capacity and therefore the risk of a pathological evolution.

Therefore, neuropsychological responses of MCI subjects to CS might serve as a potential marker for the early onset of AD. Certain features of verbal learning and memory performance on stimulation exercices may contribute to the prediction of which individuals are more likely to progress to dementia.

Conclusion

In terms of dementia prevention, learning specific cognitive strategies is a valuable means of delaying the onset of a latent dementia syndrome and of deferring the onset of symptoms. CS, therefore, with its goal of optimizing cognitive reserve capacity, can play a significant role in the treatment of MCI. This type of treatment is the more effective in that the strategies are ecological; that is, conceived in conjunction with the patient's familial and social environment as dementia tends to become manifest only when the deterioration of the patient's cognitive functions begins to have an impact on his or her daily activities.

With respect to detection, CS programs for MCI subjects can, by virtue of their goals, means, and framework, contribute to our ability to differentiate between "stable" and "at risk" subjects. Because of this, the use of CS in combination with other neuropsychological evaluations repeated over time will help lead to early diagnoses.

For these twin reasons of prevention and detection, CS occupies a critical place in the treatment of patients suffering from MCI.

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Treatment Strategies in Alzheimer's Disease

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Abstract

Intervention strategies in dementia and more specifically Alzheimer's disease (AD) are based on results from epidemiological studies on risk factors and experimental research on molecular pathogenesis of the disease and its biological substrates. Currently established treatment for AD with acetylcholinesterase inhibitors is symptomatic and cannot reverse the disease process or ameliorate neuropathological changes in the brain. Visionary interventions target more proximal events of the pathogenetic cascade of the disease like amyloid processing, tau hyperphosphorylation, apoptosis and oxidative stress. Findings from epidemiological studies have expanded our knowledge on risk as well as possible neuroprotective factors and given means to develop preventive strategies based on treatment of co-morbidity, like hypertension, or on therapy with antihyperlipidemic drugs – statins, anti-inflammatory agents or still controversial hormone replacement therapy.

Keywords: Alzheimer's disease, treatment

Increasing elderly population across the world is making operative one of the major risk factors for dementia, particularly its most common form, Alzheimer's disease. In EU estimated incidence of dementia is 824 000 persons per year[1].

Consequently, this highly prevalent chronic disease will challenge health care system and society to allocate additional funds to support not only affected individual but also caregivers. Natural course of the disease is seen as a functional deterioration over a series of clinical milestones starting with a mild cognitive impairment (MCI), progressing through the middle stages with a gradual loss of instrumental and later basic activities of daily living, emergence of behavioural problems leading to end-stage in a nursing home with a severe disability. In Sweden moderately and severely demented made up approximately 67% of the total number of dementia cases and represent 94% of the total costs utilized for dementia[2]. There is no need to put additional emphasis on the importance of intervening early during the course of the disease in order to delay or halt progression to this severe end-stage that requires expensive institutionalization.

Rationale for present and future treatments

Expanding knowledge on the etiopathogenesis of the disease and its neurobiological substrates has given means to develop treatment strategies for each level of disability, figure 1, and changed the attitude towards the disease treatment and prognosis from a passive and nihilistic towards active and optimistic.

Cascade of molecular events leading to the disease specific pathology and typical clinical expression of the disease are recognized as therapeutical targets and summarized in figure 2.

Review of intervention strategies

Treatment strategies are according to their modes of action as well as their efficacy classified into:

- symptomatic, meaning modification of the brain function without change of the disease progression;
- disease modifying, meaning intervention in the central pathogenetic event;
- preventive, meaning affecting the disease occurrence.

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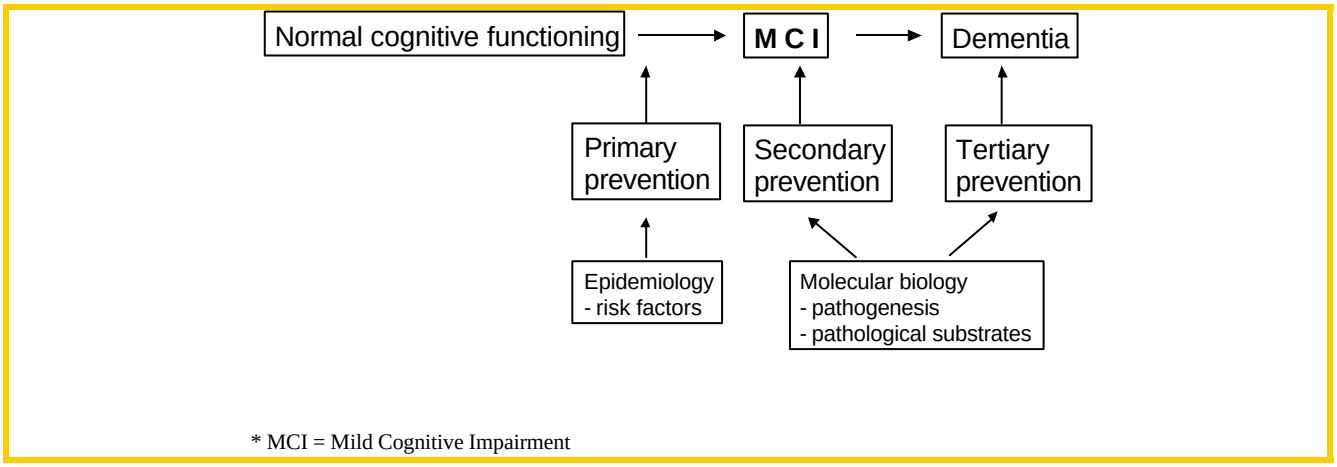

Figure 1: Intervention strategies in dementia.

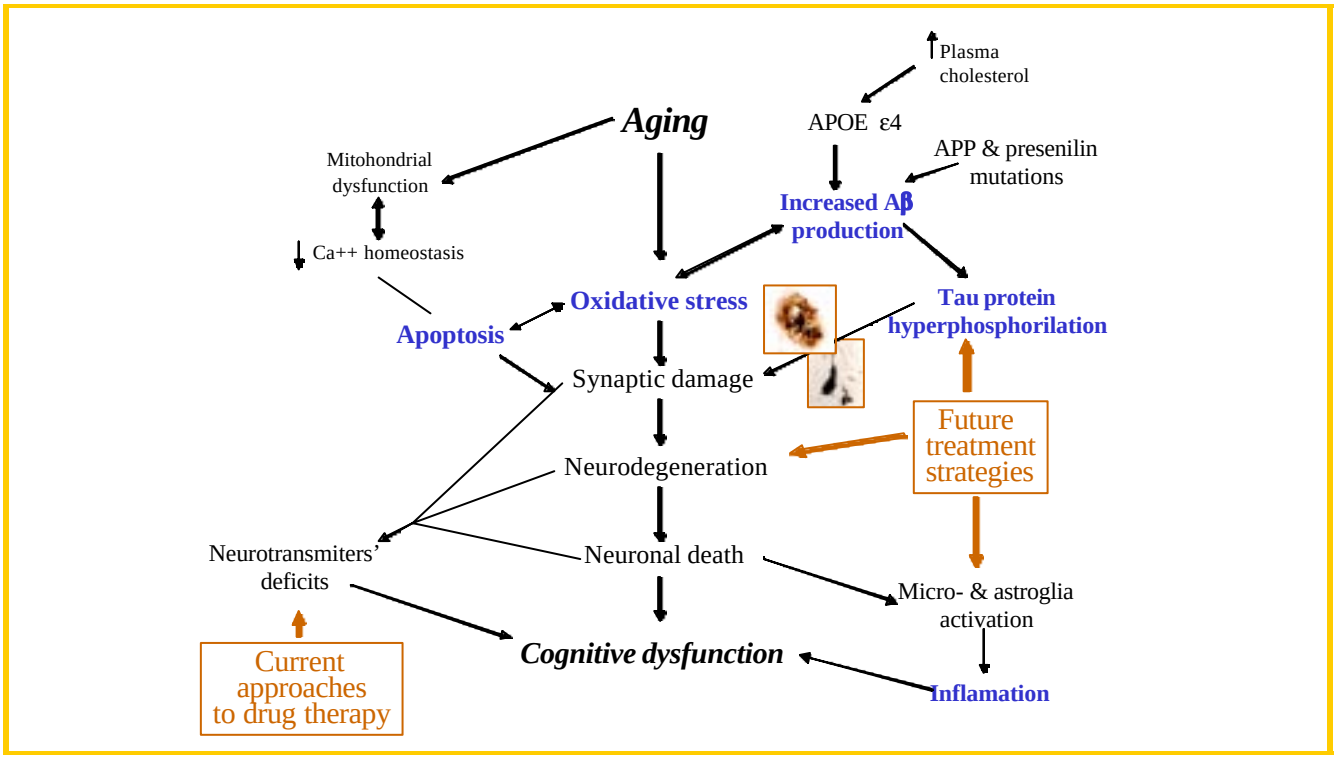

Figure 2: Molecular biology of Alzheimer's disease. Possible targets for therapeutical interventions.

a. Currently available

Current treatments operative at the clinic are mainly symptomatic and substitute transmitter deficits, i.e. acetylcholine. Efficacy of acetylcholinesterase inhibitors (AChEI) has been evaluated across three key symptom domains of AD – cognition, behaviour and activities of daily living (ADL) and short symptomatic improvements up to one year have been reported[3]. Possibilities of long-term effects via modification of amyloid precursor protein (APP) metabolism have also been considered[4] and studies designed to measure long-term efficacy of AChEI are ongoing[5]. Additional combination with neuroprotective agents like vitamins with antioxidant properties seem plausible, since oxidative stress is a central pathogenetic mechanism in various neurodegenerative diseases, including AD[6,7]. There is also more hope for the patients in moderately severe and severe stages of the disease since an antiglutamatergic drug, memantine, has been approved recently in Europe for this indication[8]. These approaches are summarized in table 1.

Table 1. Current and hypothetical treatment approaches in Alzheimer's disease.

Symptomatic	Disease modifying	Preventive
Acetylcholinesterase Inhibitors	Antioxidants (Vitamin E, Gingko Biloba)	Anti-inflammatory drugs Antihypertensive therapy
Antiglutamatergic drugs (NMDA antagonists)	NGF (gene-therapy) neotrophines?	Antihyperlipidemic agents (statins)
	Interventions in β-amyloid processing (Active* and passive** Aβ–immunization, γ & β secretase inhibitors, SAP binding inhibitors***)	Estrogen replacement therapy??
	Interventions in tau-hyperphosphorylation (glycogen synthase kinase 3β inhibitors)	

*Trial suspended, new options of immunization with a nontoxic/nonfibrillar amyloid-β are under development[9,10].
**Tested on animal model, subchronic passive immunization using the monoclonal anti-Aβ antibody[11].
***Serum amyloid P component[12].

b. Visionary

Novel treatment approaches under development or at the early stages of clinical trials are targeting central pathogenetic events that lead to the production of key pathological features of the disease: amyloid production, accumulation or aggregation; tau hyperphosphorylation with formation of neurofibrillary tangles, and apoptosis, also summarized in table 1. Nerve growth factor and neurotrophines are alternative neuroprotective approaches which promise to regulate neuronal plasticity by controling neurotransmission, connectivity and neuritic outgrowth. Growth factor gene therapy is currently in a phase I clinical trial and seems to be an effective way of delivering therapeutical substance to the most vulnerable brain regions via transplantation of patient's fibroblasts modified to secrete NGF[13].

Epidemiological studies have expanded our knowledge on risk factors for dementia, namely AD and suggested neuroprotective interventions applicable on a large scale.

Midlife vascular risk factors like high cholesterol levels and high systolic blood pressure were shown to influence late life development of cognitive impairment[14]. Therefore, treatment of co-morbid conditions could modify clinical expression of dementia, as was suggested in large retrospective epidemiological studies which showed reduced risk of developing dementia among statin users[15,16,17]. Similar reduction of AD incidence has been found in subjects exposed to anti-inflammatory drugs[18] and prevention trial with selective COX-2 inhibitor is ongoing[19]. Estrogen replacement therapy is a plausible hypothesis built on experimental data[20], but prospective observational studies were inconclusive[21]. The most recent report from Women Health Initiative on intervention trial of estrogen plus progestin therapy in prevention of chronic vascular diseases shows that overall risks exceed benefits[22].

Basic research is focusing on the development of more specific selective estrogen receptor modulators.

Implications

If proved to be successful, beneficial effects of antidementia treatments are seen as manifold. Successful interventions in early, presymptomatic stages of the disease will decrease the disease prevalence and help society to more efficiently cope with a problem and allocate more funds for multifaceted support to both patients and caregivers.

In addition, possible success of neuroprotective and disease modifying treatments will validate the existing theories on disease pathogenesis. Efficacy of similar treatments in different forms of dementia could influence clinical practice and our thinking about the neurodegenerative diseases as processes with common genetic and molecular biological background.

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Early Events of Alzheimer-Like Neurodegeneration in Anti-Nerve Growth Factor Transgenic Mice

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Abstract

Deposition of extracellular deposits of amyloid precursor protein (APP), tau hyperphosphorylation, neurofibrillary tangles and cholinergic deficits have been described in aged mice expressing anti-nerve growth factor (NGF) recombinant antibodies. We used antibodies recognising different phosphorylation-dependent epitopes of the microtubule associated protein tau, microtubule associated-protein 2 (MAP2), APP, anti-β-amyloid peptide (17-24) and AD neurofibrillary tangles to study the temporal progression of neurodegeneration in anti-NGF (AD11) mice, starting from 1 month until 15 months of age. Tau hyperphosphorylation and cellular redistribution of MAP2 were observed as early as 2 months of age at the level of the entorhinal cortex and hippocampus. At subsequent ages, phosphorylated-tau immunoreactivity extended to other cortical regions. Staining for choline acetyl-transferase in basal forebrain cholinergic neurones was reduced from 2 months onwards. Staining with anti-APP antibodies revealed extracellular deposits starting from 6 months of age and increasing with age. Anti- β-amyloid peptide antibodies revealed the presence of clusters of cells in the starting from 6-months of age. β-amyloid plaques were observed in the brain of 15-months old, but not at previous ages. Extracellular deposits were also revealed by a panel of silver impregnation methods, such as Gallyas, Campbell-Switzer and Bielschowsky. Protofilaments of PHFs were revealed by immunoelelctron microscopy in brains of 15-month old AD11 mice. Western blotting confirmed the progressive character of this neurodegeneration. We conclude that, in this comprehensive model for Alzheimer-like neurodegeneration, tau hyperphosphorylation in the entorhinal cortex, cytoskeletal changes and cholinergic deficits represent early events.

Keywords: progression, Alzheimer, insoluble phosphotau, β-amyloid deposition, cholinergic deficit

Introduction

Alzheimer's disease (AD) is a progressive neurodegeneration characterised by alterations in the processing and distribution of amyloid precursor protein (APP)[1], an abnormal post-translational processing of the microtubule associated-protein tau[2-4] and formation of insoluble intracellular tangles[5-6] Neuropathological changes include a reduction of cholinergic markers in the neocortex as well as an atrophy and degeneration of basal forebrain cholinergic neurones (BFCNs), leading to the cognitive dysfunction characterising the disease[7] . The hypothesis has been proposed that these changes are related to a loss of neurotrophic substances in the neocortex. In particular, Nerve Growth Factor (NGF) has been implicated[8], due to its well known neurotrophic effects on BFCNs[9,10] In AD, decreased NGF immunoreactivity in the basal forebrain and increased NGF and pro-NGF protein in the cerebral cortex and hippocampus have been shown[11-15], a finding that suggests a defect in the retrograde transport system for NGF[15,16]. Moreover, a failed retrograde transport of NGF has been shown in a mouse model for Down syndrome[17]. Further supporting the hypothesis of a decreased NGF transport and signalling, a decreased expression of the tyrosine kinase receptor for NGF (TrkA) has been demonstrated both in BFCNs[18-21] and neocortex[22]. NGF has also been shown to induce a recovery of cholinergic function in rodents[17,23], primates[24,25] and AD patients[26-28] However, to date, no causal relationship between the development of

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AD and NGF has been observed, also due to the lack of an experimental mouse model that would link NGF deficiency to AD-type neurodegeneration. Indeed, homozygous NGF knockout mice show an early-postnatal lethal phenotype[29] and do not allow studying the effects of NGF deficiency in the aged brain.

We have recently generated transgenic mice in which the phenotypic knockout of NGF is achieved by the expression of recombinant neutralising antibodies[30] Since the levels of transgenic anti-NGF antibodies are much higher in adult than in new-born mice, effective inhibition of NGF actions occurs in the adults only. Aged anti-NGF (AD11) show a severe cholinergic and behavioural impairment, extensive neurofibrillary pathology linked to the protein tau, extracellular deposits of APP[31] and β-amyloid plaques[32]. Moreover, AD11 mice show a significant impairment of cortical synaptic plasticity[33]. On the whole, this phenotype has striking similarities to AD, and AD11 mice represent therefore a comprehensive model for the sporadic form of the disease[31].

In this paper we investigated the temporal progression of neurodegeneration in AD11 mice. The results show that in AD11 mice tau hyperphosphorylation and cholinergic deficits represent early events and that entorhinal cortex is the first neocortical area affected by the neurodegeneration.

Materials and methods

Production of transgenic mice. The pronucleus of single-cell fertilised C57BL/6 x SJLF2 hybrid mouse eggs were injected with pcDNAI-neo/VKαD11HuCK and pcDNAI-neo/VHαD11HuCγ plasmid fragments containing the transcriptional unit of the light or heavy chain genes of the chimaeric antibody αD11 [34]. Crossings designed to obtain AD11 mice and analysis of transgenic mice were performed as previously described [30]. Mice homozygous for the light chain (VKαD11 mice, lines A and B) and mice homozygous for the heavy chain (VHαD11, lines C and D) were obtained. The breeding between two different lines of VKαD11 mice and VHαD11 mice gave rise to independent family of mice. Family 1 is characterized by a value of transgenic antibody in the brain equal to 70 ± 0.5 ng/mg brain. The AD-like neurodegeneration characterizing this family was previously described [31, 32]. Mice belonging to family 2 show a level of transgenic antibody equal to 140 ± 0.1 ng/mg of brain tissue. The experiments described in article were performed on mice from both family 1 and family 2. Mice expressing only the VHαD11 chain were used as control mice. All experiments were performed according to the European Community Directive on animal care.

Histochemistry and Immunohistochemistry. The presence of neurofibrillary pathology was assessed in 2-, 6- and 15- months old AD11 mice using the Bielschowsky and Gallyas silver impregnation methods[35,36]. Amyloid deposits were revealed using Campbell-Switzer silver impregnation method (Campbell, Switzer and Martin, 13th Annual Meeting Society for Neuroscience, abstract 678, 1987).

Immunohistochemical experiments were performed on transgenic controls (VH only) and double transgenic (VH and VK) AD11 mice at different postnatal ages, i.e. 1-, 1.5-, 2-, 4- , 6-, 8-, 10-, 12- and 15-months. Mice were anaesthetised with 10.5% chloral hydrate/saline (8 µl/g body weight) and transcardially perfused with 4% paraformaldehyde (PFA) in phosphate buffered saline (PBS). Brains were removed, postfixed for two hours at 4°C, cryoprotected in 30% sucrose/PBS for 1 hour and then in 20% sucrose/PBS overnight. Coronal sections (40 µm thick) were cut using a freezing sliding microtome (Leica, Heerbrugg, Switzerland) and collected in a six-well culture plate in 4% PFA/PBS. Sections were processed for detection of different antigens using the peroxidase conjugate mouse-on-mouse immunodetection kit (Vector Laboratories, Burlingame, CA). The following primary antibodies were used: anti-choline acetyl-transferase (ChAT; Chemicon, Temecula, CA), anti-TrkA (MNAC13[37]) anti-microtubule associated protein 2 (MAP2, clone AP-20; Sigma, St Louis, Mo) and anti-phosphorylation dependent tau antibodies. In particular, we examined the phosphorylation state of the microtubule associated-protein tau in AD11 mice using antibodies specific for distinct phosphorylated epitopes of tau: AT100, recognising Ser-212 and Thr-214 phosphorylation sites, mAbs AT180 and AT270 identifying Thr-231 and Thr-181 phosphorylated epitopes, respectively, mAb AT8 recognising phosphoSer-202 and phosphoSer 205, mAb PHF-1 identifying phosphoSer-396 and phosphoSer 404 sites. Clones AT8, AT100, AT180 and AT270, were purchased from Innogenetics (Gent, Belgium), while the clone PHF-1 was kindly provided by P. Davies (Albert Einstein College of Medicine, Bronx, NY).

Anti-amyloid precursor protein APP (clone 2.F2.19B4,Chemicon), anti-β-amyloid 17-24 (clone 4G8, Senetek, Maryland Heights, MO), against the NH_2-terminus of Aβ (R3660, kindly provided by Prof. Schettini and Dr. Russo, University of Genova, Italy).-and anti-tangle antibody (clone MIG-T4, Innogenetics) were used to identify extracellular deposits of APP and β-amyloid and neurofibrillary tangles, respectively. To verify the specificity of anti-β-amyloid immunolabelling, the primary antibody was preadsorbed for 1 hour against a fragment of β-amyloid protein including residues 12-28 (Research Biochemicals International, Natick, MA).

Double immunohistochemistry was performed using the anti-β amyloid 17-24 antibody and biotinylated anti-human IgG (K light chains or heavy chain specific) (Vector Labtek). The reaction was revealed tetramethylrhodamine isothyocianate (TRITC) conjugated anti-mouse (Sigma) or antibodies and extravidin FITC conjugate (Sigma).

Parallel sections from AD11 transgenic and age-matched transgenic control (VH only) mice were collected and processed simultaneously. A set of experiments was

performed by incubating in parallel mouse and human sections. The latter were obtained from AD human brains supplied by the Netherlands Brain Bank.

Neuronal loss was evaluated by using cresyl violet staining or the In Situ Cell Death Detection Kit (Roche Diagnostics). Cell counts were performed as described previously[31].

Quantitative stereology on BFCNs. Anatomical boundaries used to define the basal forebrain were the corpus callosum, for the dorsal aspect, the ventral surface of the brain and, laterally, a line passing medial to the rostral limb of the anterior commissure. The rostral boundary was determined by a plane passing through the rostral genu of the corpus callosum and the caudal boundary was coincident with a plane passing through the first section containing the anterior commissure. The diagonal band nucleus was distinguished from the medial septum using a plane passing ventral to the rostral limb of the anterior commissure. All anatomical references were taken from Franklin and Paxinos[38]. The volume of the BF was calculated using the method of Cavalieri[30, 39, 40].

Estimation of the total number of ChAT-positive neurones was achieved using the optical fractional method[38,41] . High resolution images (60 x) were acquired on a Zeiss microscope (Carl Zeiss, Oberkochen, Germany) equipped with a CCD camera and displayed by using the Optimas 6.1 analysis program (Optimas Corp., Bothell, WA). The total number of neurons was evaluated as described previously[30] Volume and total number of ChAT positive-cells were evaluated using at least 3 animals for each group. Statistical analysis was performed using a two tail T-test.

Amyloid burden. The plaque density was quantified on 40 µm brain sections and reacted with the anti-APP and anti β-amyloid antibodies at 15 months of age. The analysis was blindly performed using the method described by Schenk *et al*. [42] and the image analysis system described above. After image capturing, the entorhinal cortex was outlined and the total pixel area occupied by this cortical structure was determined. A monochromatic-based threshold was fixed to select pixels corresponding to the immunolabelled plaques. The percentage of the brain region occupied by labelled pixels was calculated. For this analysis, four sections for each animal were used (n = 7 for each experimental group).

Western blot analysis of tau and amyloid proteins. For Western analysis, brains were homogenised with a polytron homogenizer (Janke & Kunkel GmbH, Hamburg, Germany) in 5 ml/g of ice cold extraction buffer (50 mM Tris-HCl, pH 7.5, 50 mM EDTA, 250 mM spermidine, 1mM phenylmethylsulfonyl fluoride (PMSF), 1 mM iodoacetamide, 10 µg/ml leupeptin, 1 µg/ml aprotinin, 4µg/ml soybean trypsin inhibitor, 10µg/ml turkey egg white inhibitor). Homogenates were centrifuged at 44,000 X g for 30 minutes at 4°C and supernatants were analysed by SDS-PAGE (10% and 15% polyacrylamide gels, as appropriate) and Western blot using PHF-1, AT8 and anti-APP as primary antibodies. The intensities of the immunoreactive bands were quantified and analysed using the NIH image analysis program (NIH Scion Image, Scion Corp., Frederick, MD) after normalising for the protein content, evaluated by the intensity of the tubulin band with mAb YOL-1 (kindly provided by C. Milstein, Medical Research Council Laboratory of Molecular Biology, Cambridge, UK).

Tau protein solubility in the brain of aged AD11 mice. To study the solubility of tau protein in aged AD11 mice, brains from control and transgenic mice were processed as described before [43]. Tissue was homogenised in 2ml/g of ice-cold reassembly high salt buffer [RAB Hi-salt: 0.1 M morpholineethanesulfonic acid (MES), 1 mM EGTA, 0.5 mM $MgSO_4$, 0.75 M NaCl, 0.02 M NaF, 1mM phenylmethylsulfonyl fluoride, protease inhibitor cocktail (Complete mini, Roche Diagnostics), 100 mM EDTA, pH 7.0] and centrifuged at 50,000 x g for 40 min. at 4°C. The supernatants were boiled for 5 min, chilled on ice and re-centrifuged at 10,000 x g for 20 min. at 4°C. To obtained tau insoluble fractions, pellets were extracted with 1 M sucrose/RAB (0.1 M MES, 1 mM EGTA, 0.5 mM $MgSO_4$, pH 7.0) at 50,000 x g for 20 min at 4°C. Supernatants were discarded and the obtained pellets were re-homogenised in 1ml/g radioimmunoprecipitation assay (RIPA) buffer (50 mM Tris, 150 mM NaCl, 1% Triton-X 100, 5 mM EDTA, 0.5 % sodium deoxycholate, 0.1% sodium dodecylsulfate, pH 8.0) and centrifuged at 50,000 x g for 20 min at 4°C to obtain RIPA soluble fractions. Pelletes were re-extracted in 70% formic acid (FA), lyophilised and reconstituted in gel loading buffer. Western blots of the RAB soluble-, RIPA soluble- and FA-soluble fractions were performed using AT8 as primary antibody.

Immunoelectron microscopy on brain extracts from aged AD11 mice. To assess the presence of paired helical filaments in brain of aged AD11 mice, we used a procedure described before by Goedert *et al* [44]. Entorhinal cortices from AD11 mice, control mice, human non demented subjects and from human AD patients were homogenized in 10 ml/g of 10 mM Tris-HCl (pH 7.4), 0.8 M NaCl, 1 mM EGTA and 10% sucrose plus a cocktail of proteinase inhibitors (Complete Mini, Roche diagnostics). The homogenate was spun for 20 min at 20,000 x g at 4°C. The pellet was rehomogenized in 5ml/g of the same buffer and recentrifuged. Both supernatants were combined and brought to 1% N-lauroylsarcosinate and incubated for 1 hour at room temperature while shaking. Following 1 hour spin at 100,000 x g, the pellets were resuspended in 50 mM Tris-HCl (0.2 ml/g). A droplet of the resuspended pellet was placed on carbon-coated copper grid (400 mesh) and allowed to evaporate partially. For antibody labeling, grids were placed on a drop of 0.1% gelatin in PBS for 5 min. After blotting the excess of solution, grids were then placed on a solution of primary antibody for 1 hour at room temperature. MAb AT8 was used a t a 1: 2 dilution in PBS. The reaction was completed by placing the grids on

secondary antibody conjugated to gold particles for 1 hour at room temperature. After washing, the grids were stained with a few drops of 1% lithium phosphotungstate and allowed to dry. Micrographs were recorded on a Philips scanning electron microscope, at an operating voltage of 80 KV and at a nominal magnification of 56,000 x.

Neuronal loss. Counts to determine the presence of neuronal cortex were performed in a brain region with well defined boundaries and within a selected neuronal population that could be identified by Cresyl violet staining. For this reasons we chose to stain brain sections with Cresyl violet and to perform counting of layer V large pyramidal neurons characterizing the primary visual cortex. The boundaries of the primary visual cortex were described before [45]. Stereological counts were performed from bregma - -2.70 to bregma –3.28.

Results

We have previously described the generation of AD11 transgenic mice[30], in which the heavy and the light chains of the AD11 recombinant antibody anti-NGF αD11 were expressed under the control of the human cytomegalovirus early gene promoter. In a subsequent study we reported the appearance of an AD like neurodegeneration in aged AD11 mice, obtained by crossing line A with line D (family 1)[31] The neurodegenerative phenotype in AD11 mice is characterised by cholinergic deficit, tau hyperphosphorylation, neuronal loss, neurofibrillary tangles, extracellular deposits of APP[31] and β-amyloid plaques[32]. Six months old anti-NGf mice show a marked impairment of cortical synaptic plasticity[33]. Adult anti NGF mice also reveal a progressive atrophy of skeletal muscles, which is highly reminiscent of inclusion body myositis (IBM)[46], a muscular syndrome frequently associated to AD[47].

In this study we increased the number of immunochemical markers analysed (including antibodies to β-amyloid fragment of APP) and extended our analysis, both in younger and aged mice, belonging to a second independent family (family 2), to study the temporal progression of the neurodegeneration and describe the age-dependency of different neuropathological markers. We examined a total of 120 mice in the age range from 1 to 22 months (62 AD11 mice and 58 transgenic control mice, matched for age and sex). Approximately equal numbers of each sex were examined. At all ages analysed, all markers characterising the phenotype were consistently altered in each mouse analysed, with little inter-individual variability. The data obtained in this study are summarized in table 1.

Neuronal loss

At 1, 1.5 and 2 months of age no neuronal loss was observed in the cortex of AD11 mice, as determined by cresyl violet counts. A localised neuronal loss was observed in the entorhinal cortex of 6 month-old AD11 mice, reaching a 25% of decrease. Diffuse neuronal loss was observed in the entorhinal cortex of 15 month-old AD11 mice with an average decrease in the number of cresyl violet stained neurons equal to the 34%. A detailed, neurostereological analysis was performed at the level of the visual cortex. Neuronal counts revealed that the number of large pyramidal cells in the primary visual cortex of AD11 mice started to decrease from 8 months of age (Fig. 1A). Neuronal loss was progressive and reached its peak at 15 months of age, when almost 40% of pyramidal cells in cortical layer V was lost. No neuronal loss was detected in age-matched control mice (Fig. 1A).

DNA fragmentation, a sign of putative apoptosis, was observed only in the cortex of 15 month-old AD11 mice, as previously reported[31] and not in younger animals (data not shown).

Basal forebrain cholinergic deficit and neuronal loss in AD11 mice

The cholinergic deficit in 2 month old[30] and aged[31] AD11 mice is characterised by a decrease in the number of ChAT-positive neurons. We now show that in 1 month and 1.5 month-old mice the number of ChAT-positive BFCNs was not statistically different from that of age-matched control mice. From 2 months of age onwards it progressively declined, reaching a plateau at 6 months (Fig. 1B).

In 2 months old AD11 mice, the observed reduction in the number of ChAT-positive neurons is paralleled by a concomitant reduction in the number of TrkA-positive neurons (Fig. 1C), while the number of p75-positive neurons remains the same (Fig. 1D). At this age, most cells in the BF of AD11 mice appear viable from a morphological point of view, and therefore the effect of anti-NGF antibodies is most likely a modulation of the expression of ChAT and TrkA. On the other hand, in the BF of 6-months old AD11 mice, TrkA-positive (Fig. 1C) and p75-positive cells (Fig. 1D) are virtually absent, which correlates with the fact that almost 50% of the BFCNs are picknotic (Fig. 1E).

Temporal progression of tau hyperphosphorylation in AD11 mice

In the cortex and hippocampus of AD11 mice, mAb AT100 labels many neuronal perykaria at all examined ages (Fig. 2 A-D; E, negative control without primary antibody) and the pattern of staining and number of labelled cells was not different from that in control mice (data not shown).

At 1-2 months of age the distribution of AT180 immunolabelling was confined to axons in the white matter (data not shown) while cortical and hippocampal neurones were devoid of labelling (Fig. 2F). However, in the cortex of 6 month-old AD11 mice few non-neuronal cells and dystrophic dendrites appeared to be labelled by AT180 (Fig. 2G). The

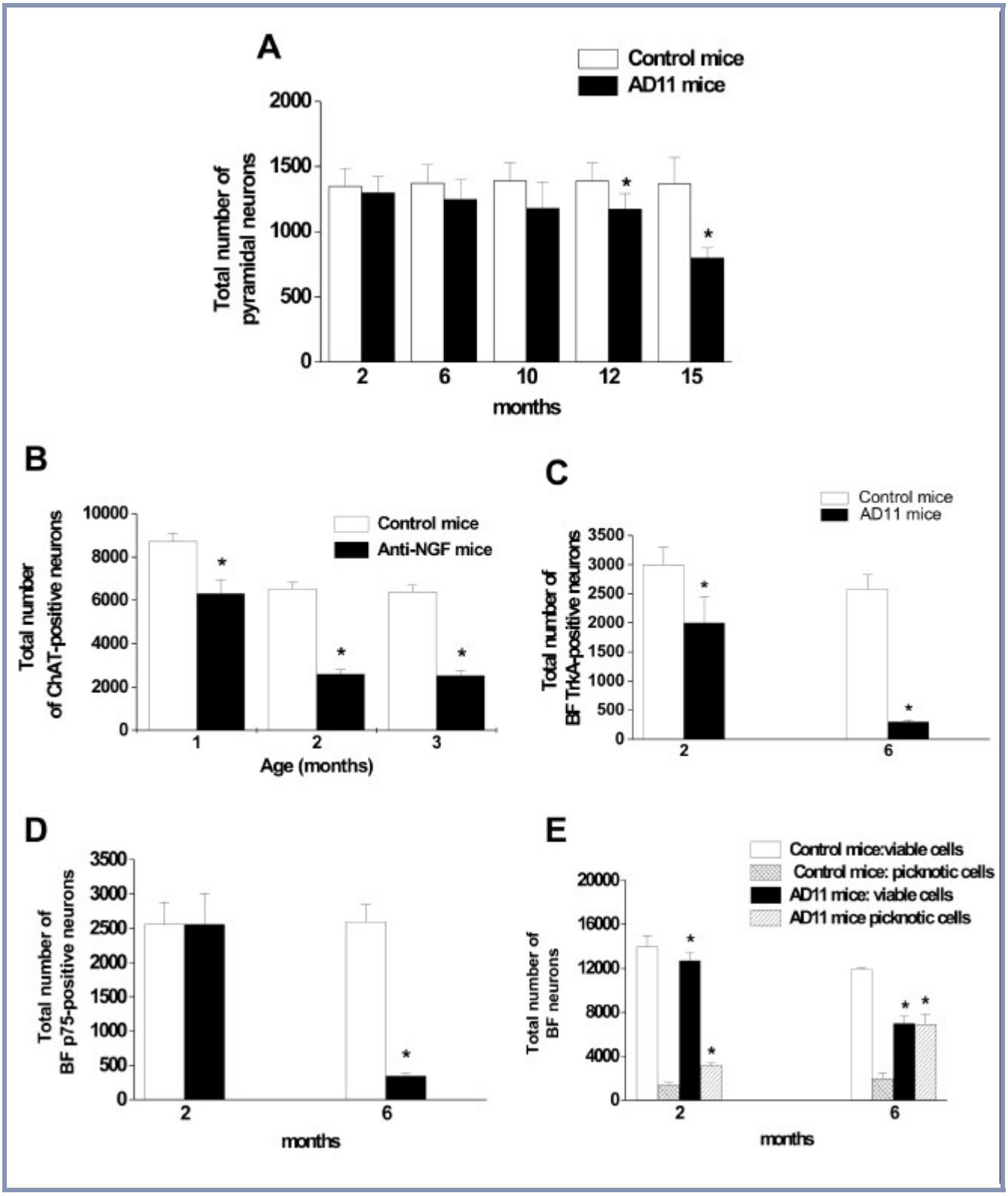


Figure 1. **(A)** Neuronal loss in visual cortex of AD11 mice.

(B) Basal forebrain cholinergic deficit in AD11 mice. Histogram shows the total number of ChAT–positive neurons in the basal forebrain of AD11 and control mice at different postnatal ages.
The total number of **(C)** TrkA-positive neurons and **(D)** p75 positive neurons in the BF is progressively decreasing in AD11 mice and **(E)** it is accompanied by a high number of picknotic cells.* $P < 0.01$. Values represent the mean ± SEM (n = 6 for each group of animals).

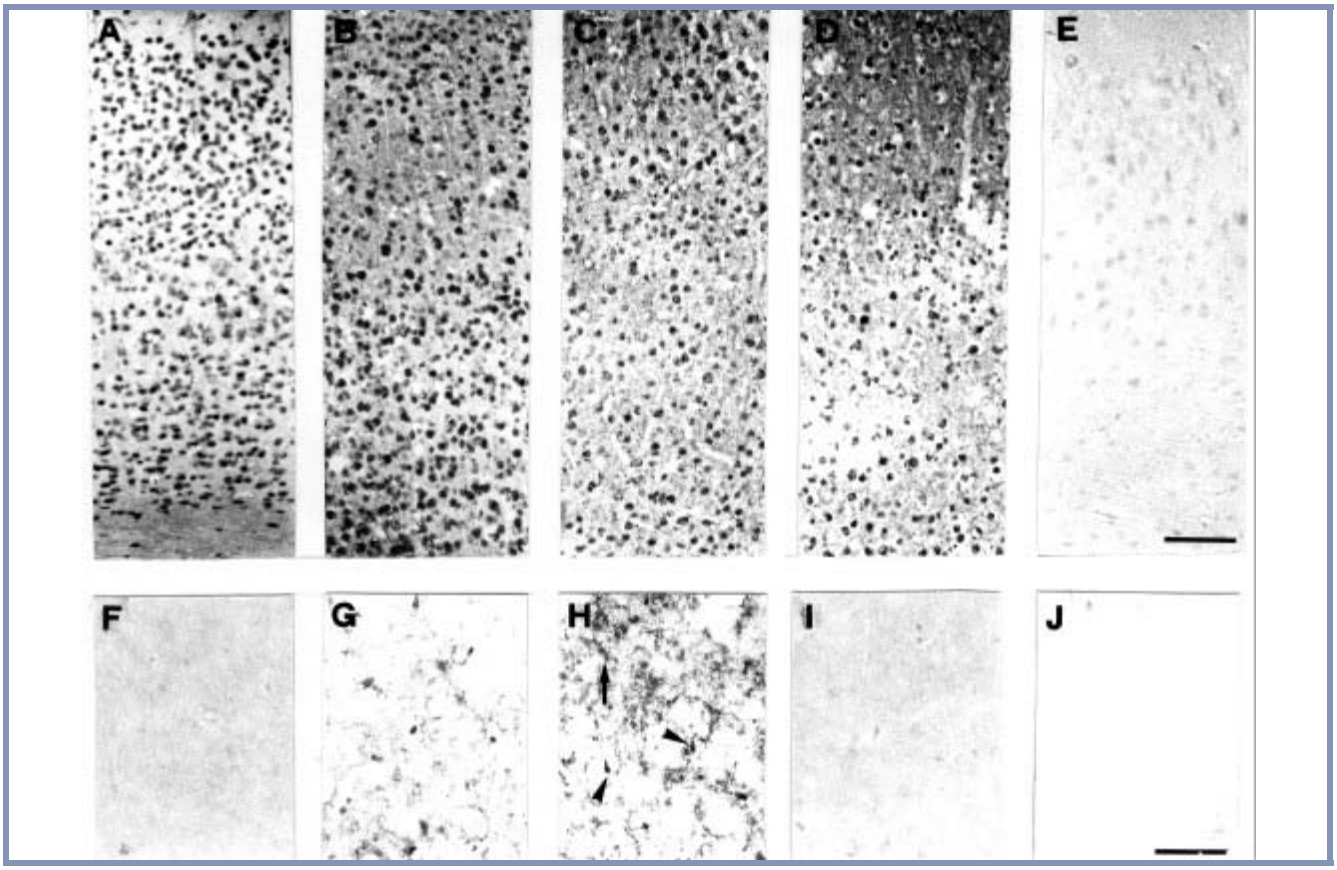


Figure 2. AT100 and AT180 immunolabelling in cerebral cortex of AD11 mice. AT100 immunolabelling in: **(A)** 1.5 month-old anti-NGF mice **(B)** 2 month-old anti-NGF mice **(C)** 6 month-old anti-NGF mice **(D)** 15 month-old anti-NGF mice **(E)** negative control (primary antibody omitted). AT180 immunolabelling in: **(F)** 2 month-old anti-NGF mice **(G)** 6 month-old anti-NGF mice **(H)** 15 month-old anti-NGF mice **(I)** 15 month-old control mice , **(J)** negative control (primary antibody omitted) Scale bars in A-E = 150 µm; in F-J = 75 µm.

number of positive cells and the intensity of the staining increased in sections from 15 months old mice (Fig. 2H). No cell bodies or dendrites appeared to be labelled in aged-matched control mice (Fig. 2I) or in sections where the primary antibody was omitted (Fig. 2J).

MAb AT270 displays the same labelling pattern in the cortex of AD11 and control mice until 6 months of age.In 1 month-old AD11 and control mice, AT270 labelled neuropil and cells in cortical layer II/III (Fig. 3A). The intensity of staining of labelled cell bodies increased in 1.5-months old mice while that of neuropil decreased (Fig. 3B), both of which disappeared in 2-month old mice (Fig. 3C). At 6-months of age, scattered cells appeared to show a somatic labelling in cortical layers VI and II from AD11 mice (Fig. 3D), and their number increases with age. At 15-months of age, many cortical neurons were AT270-positive and labelling in axons disappeared (Fig. 3E). In 6 (data not shown) and 15 month-old control mice (Fig. 3F) the overall labelling of cells and neuropil was much weaker.

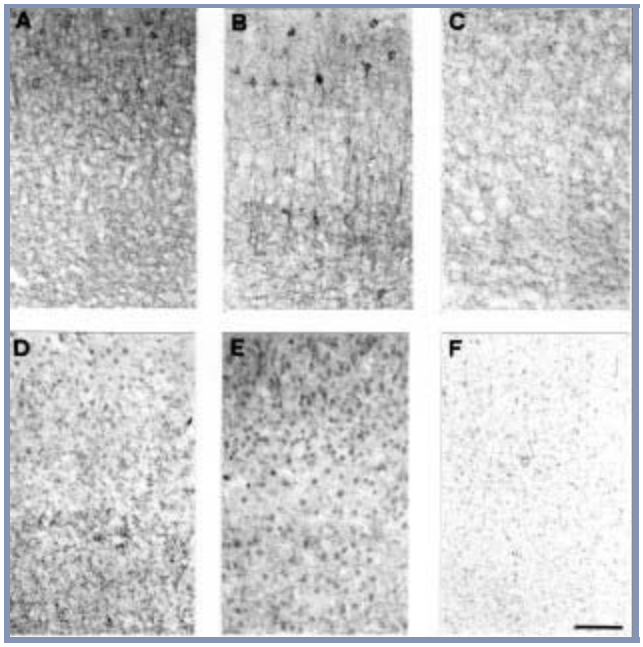


Figure 3. AT270 immunolabelling in the cortex of AD11 mice. AT270 immunolabelling in cerebral cortex of AD11 mice at **(A)** 1 month, **(B)** 1.5 months and **(C)** 2 months of age. No difference was observed when compared to control mice. Starting from 6 months of age in AD11 mice a specific labelling shows up in neurons of layers VI and II/III **(D)**. The number of these positive cells increases in 15 month-old AD11 mice **(E)**. with respect to control mice **(F)**. At both ages no labelling is observed in age-matched control mice (F, 15 months old control mouse). Scale bar 100 µm.

In the hippocampus, at 1-10 months of age AT270 labelling was shown only in neuronal processes of both AD11 and control mice (data not shown), while it highlights a distinct pattern from 12 to 15 months of age in AD11 mice where many neurons were labelled (Fig. 4A). In age-matched control mice, only thin neuronal processes were labelled (Fig. 4B).

The same cellular pattern was observed in the hippocampus of AD11 mice using the anti-phosphorylated tau AT8 antibody (Fig. 4C), while in control mice AT8 labelling was confined to axons of hippocampal neurons (Fig. 4D). However, the temporal progression of AT8 labelling in the hippocampus was different from that observed using mAb 270, starting to appear from 4 months of age. Omission of the primary antibody during incubation allowed verifying the specificity of immunolabelling (Fig. 4 E,F).

The spatio-temporal distribution of AT8-labeled neurons in the cerebral cortex of AD11 mice was particularly revealing. At 1 month of age, AT8-positive neurons were revealed throughout the cerebral cortex of both AD11 (Fig. 5 A) and control mice (Fig. 5B) and their number decreased at 1.5 months of age both in AD11 (Fig.5C) and in age-matched controls (data not shown). However, from 2 months onwards the staining in AD11 mice changed pattern of distribution and increased in intensity. At 2-months, AD11 mice showed a high number of AT8-positive neuronal bodies in the entorhinal cortex (Fig.5D). No staining was seen in the entorhinal cortex from control mice (Fig. 5E). Only faint and scattered cells were observed in the parietal cortex of 2-month-old AD11 mice, while axons were more intensely labelled (Fig. 5F). By 6 months, the AT8 labelling extended throughout the cortex of AD11 mice (entorhinal cortex: Fig. 5G; parietal cortex: Fig. 5H), while no labelling was observed in age-matched controls (Fig 5I). At this age, the staining was distributed in cell bodies as well as in dendrites (Fig. 5G, H). The arrows in Fig. 5G indicate the AT8 labelling in

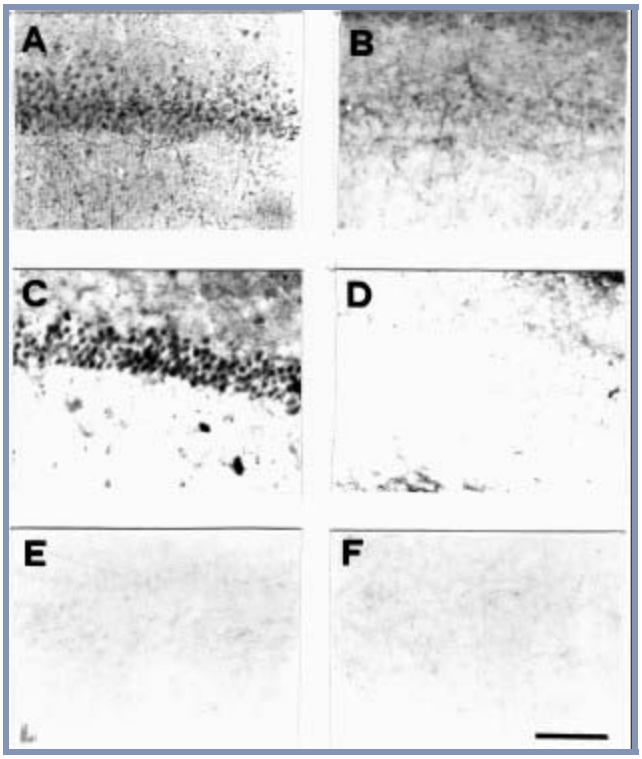


Figure 4. AT270 and AT8 immunollabeling in the hippocampus of aged AD11 mice. AT270 **(A,B)** and AT8 **(C,D)** antibodies labels pyramidal cells in hippocampal CA1 region in 15 month-old AD11 mice (AT270:A; AT8: C). **(E,F)** In age-matched control mice AT270 **(B)** and AT8 **(D)** antibodies label only axons. **(E,F)** Negative controls for immunohistochemistry (primary antibody omitted). Scale bar 100 µm.

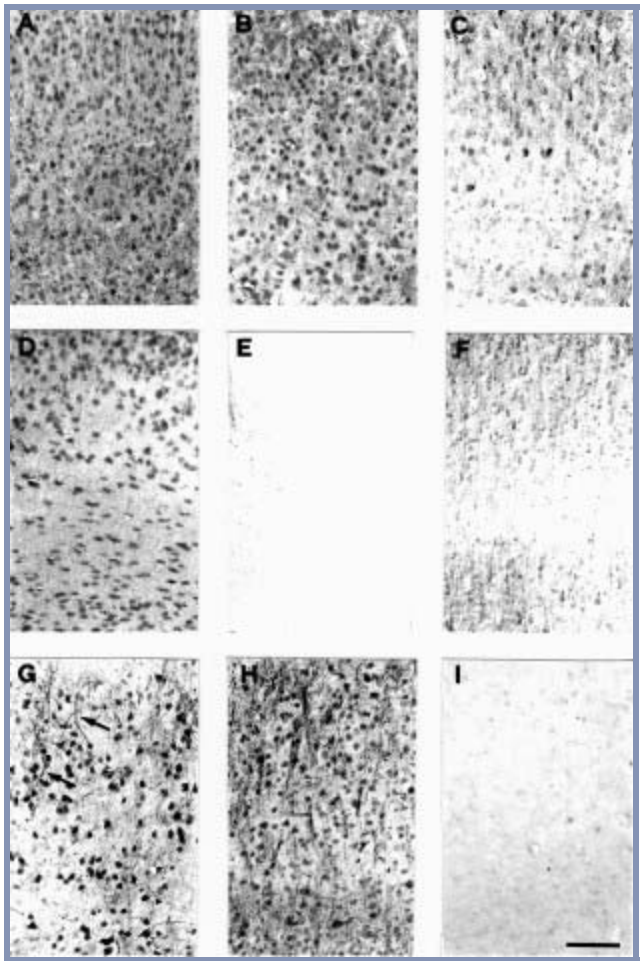


Figure 5. Temporal progression of AT8 immunolabelling in AD11 mice. At 1 month of age, AT8 antibodies stain neurons in all cortical layers of the entorhinal **(A)** and parietal **(B)** cortices of AD11 mice. In both cortices, the labelling decreases at 1.5 months of age **(C)**. No difference is seen with control mice. At 2 months of age, AT8-positive neurons are observed in the entorhinal cortex **(D)**, while only a few cells are faintly labelled in the parietal cortex **(F)**. In age-matched control mice there is no labelling in neuronal bodies **(E)**. In 6 month-old mice most neurons of the entorhinal cortex **(G)** express AT8 in their cell body and many of them also in dendrites (arrows). At this age, the parietal cortex **(H)** shows labelling both in neuronal perikarya and dendrites. No labelling **(D)** was observed neuronal bodies or dendrites of age-matched control mice **(I)**. Scale bar 100 µm.

neuronal dendrites. At 12 and 15 months of age, many AT8-positive neurons showed a flame-like shape, filled with tangle-like inclusions (Fig. 6A), similar to those observed with the same antibody in human brain sections from AD patients (Fig. 6B). Dystrophic neurites and tau-positive fibrous material observed in the extracellular space in sections from AD11 mice was very similar to what is shown in AD human brains (Fig. 6A,B).

A completely overlapping time course and labelling pattern was seen using the monoclonal antibody PHF-1 (data not shown), which recognizes tau phosphorylated at residues 396 and 404 [48].

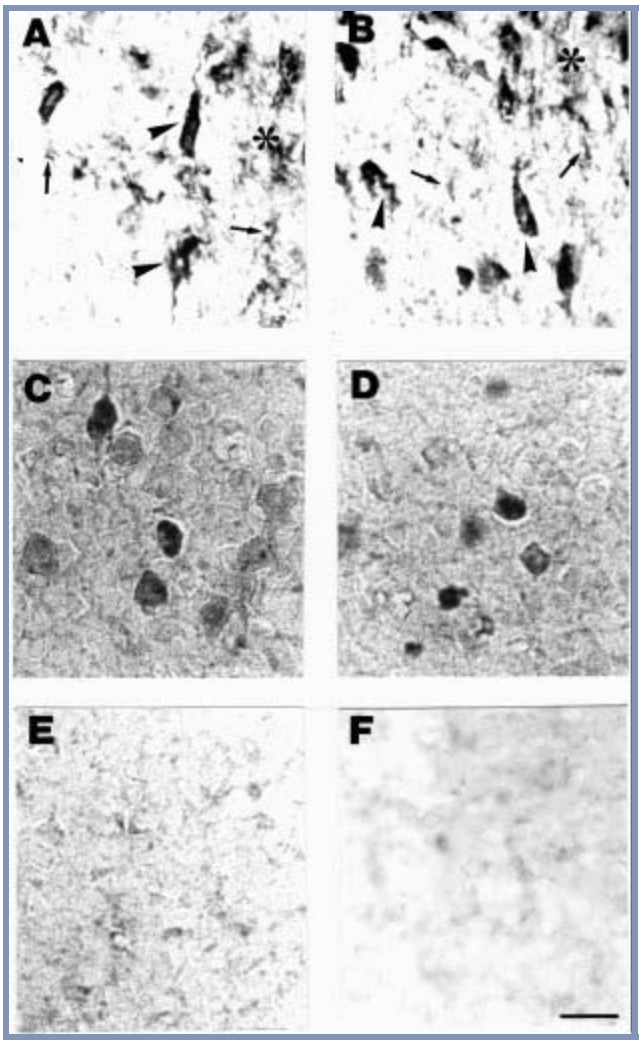


Figure 6. AT8 and NFT200 labelling in the cortex of AD11 mice and AD human patients. In 15 month-old mice **(A)**, AT8 antibodies label dystrophic neurites (arrows), extracellular neurofibrillary deposits (asterisks) and tangle-like structures (arrowheads). The pattern is similar to that observed in AD human brains **(B)**. **(C)** Anti-tangle antibody NFT200 stains tangles in cerebral cortex of AD11 mice. The morphology is similar to the tangles revealed by the same antibody in AD human brain **(D)**. No staining by NFT200 is shown in the cortical area from transgenic control mice **(E)** or omitting the primary antibody during incubation **(F)**. Scale bar 50 µm.

The presence of tangle-like assemblies in the cortex of AD11 mice was further assessed with monoclonal antibody NFT200, that recognizes an epitope in the neurofilament protein associated to neurofibrillary tangles in human AD brains. MAb NFT200 gives a labelling in the entorhinal cortex of 12 months old AD11 mice (not shown) and throughout the cortex at 15 months of age (Fig. 6C), very similar to that observed in human AD brains, as shown in Fig. 6D [49]. The appearance of the aberrant structures labelled by mAb NFT200 in the cortex of AD11 mice (Fig. 6C) was very similar to that observed in human AD brains (Fig. 6D). In the hippocampus, at 15 months of age, few cells of the CA1 region were also labelled with mAb NFT200 (data not shown), while other hippocampal areas did not show positive cells. These NFT200-positive cells were not observed in transgenic controls (Fig. 6E) or when the primary antibody was omitted (Fig. 6F).

Temporal progression of MAP-2 abnormal compartmentalisation in AD11 mice.

The state of the neuronal cytoskeleton was further analysed by investigating the subcellular distribution of the microtubule associated protein MAP-2 by immunohistochemistry.

At 1 and 1.5 months of age no difference in MAP-2 labelling was observed between control and AD11 mice (data not shown). In pyramidal neurons of the cortex of 2 month-old control mice, MAP-2 immunoreactivity was widely distributed throughout the dendritic length (Fig. 7A). At this age the number of MAP-2-positive dendrites in the cortex of AD11 mice started decreasing (Fig. 7B). Between 4 and 15 months of age, in AD11 mice the number of MAP2-labelled dendrites continued to decrease and the labelling progressively became fragmented and localised to the proximal part of dendrites (Fig.7 C, D, 6 month-old control and AD11 mice; Fig. 7E,F 15 month-old control and AD11 mice), indicating that major reorganization of the neuronal cytoskeleton is an early event in the progression of neurodegeneration in AD11 mice.

Temporal progression of amyloid deposition in the brain of AD11 mice

We have recently shown that the brain of aged (15 month-old) AD11 mice contains deposits of APP [31] and of β-amyloid [32]. The temporal progression of the extracellular deposition of amyloid material was now investigated with antibodies against APP and against β-amyloid. The anti-APP antibody recognizes the carboxyl terminus of APP and in human AD brains reveals full length and cytoplasmic fragments of APP. At 1, and 1.5 months of age no labelling for APP was shown in the brain of AD11 and control mice (data not shown). At 2 and 4 months of age, the walls of some cerebral vessels was intensely labelled (Fig. 8A) with

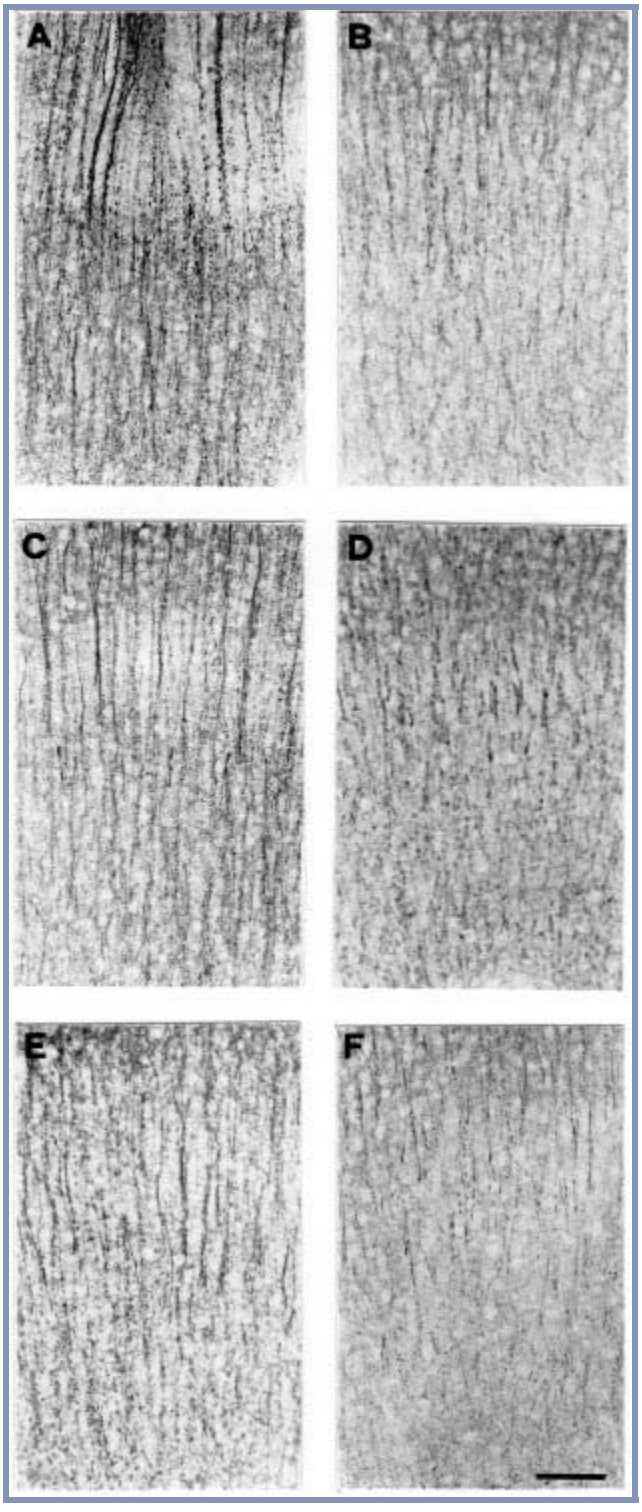


Figure 7. MAP-2 abnormal distribution in AD11 mice.
At 2 **(A)**, 6 **(B)** and 15 **(E)** months of age anti-MAP-2 labels the full length of cortical dendrites in control mice. In AD11 mice, a reduction of the number of labelled-dendrites and a re-distribution of the staining is observed. The decrease in staining starts at 2 months of age **(B)** and proceeds with ageing (**D, F**: 6 and 15 months of age, respectively). Scale bar = 100 µm.

respect to those of age-matched control mice (Fig. 8B). At 6 months of age, small and compact APP-immunoreactive deposits were also seen in the cortex (Fig. 8E). At 15 months of age, the number of APP-positive extracellular deposits increased in AD11 mice, with a burden, in the entorhinal cortex, of 3.6 % at 6 months of age and of 19% at 15 months of age. The burden of APP deposits in the same brain region was equal to 0.01% and 0.5% in control mice at 6 and 15 months of age, respectively.

The presence of β-amyloid peptide fragments from APP processing was detected in the brains of AD11 mice with monoclonal antibody 4G8, recognizing residues 17-24 of β-amyloid. At 6 months of age, mAb 4G8 revealed a strong labelling in the cerebral vasculature (Fig. 8 C,D) and no labelling whatsoever of the hippocampal region, nor of other brain regions (data not shown). At this age, Aβ was detected only in cell clusters in the hippocampus, by using an antibody directed against the NH_2-terminus of Aβ (Fig. 8H). Between 1 to 12 months of age, no extracellular labelling was observed (data not shown). On the other hand, at 15-months of age, β-amyloid plaques were observed in the cortex and hippocampus (Fig. 8F) of AD11 mice. No labelling was observed in control mice (Fig. 8G), or in presence of an excess of the corresponding peptide (data not shown). Higher magnification views β-amyloid deposits surrounded by positive cells is shown in Fig. 8I. For comparison, a plaques from AD brain section, revealed with mAb 4G8, is shown in Fig. 8J.

Detection of extracellular amyloid and neurofibrillary deposits by silver impregnation methods.

Brain sections from 2-, 6- and 15-months old anti NGF and age matched control mice were stained following the modified silver impregnation method by Bielschowsky. The concomitant presence of fibrillary argyrofilic round-shaped deposits and dystrophic neurites (Fig. 9A) was demonstrated in brain sections from 15-months old anti NGF mice, but not in those from control (Fig. 9B) and from 2- (Fig. 9C) and 6- (Fig. 9D) months old AD11 mice.

Gallyas silver impregnation method revealed the presence of extracellular neurofibrillary deposits and tangles like filled cells in 15-months old AD11 mice (Fig. 9E) but not in aged-matched control (Fig. 9F) and in 2- or 6 months AD11 mice (Fig. 9G and H, respectively).

Campbell- Switzer staining detected extracellular deposits encircled by black-stained cells in 15 months old AD11 mice (Fig. 9I) that were present also in 2- (Fig. 9J), 6- (Fig. 9K) months old AD11 mice and in 15-months old control mice (Fig. 9L).

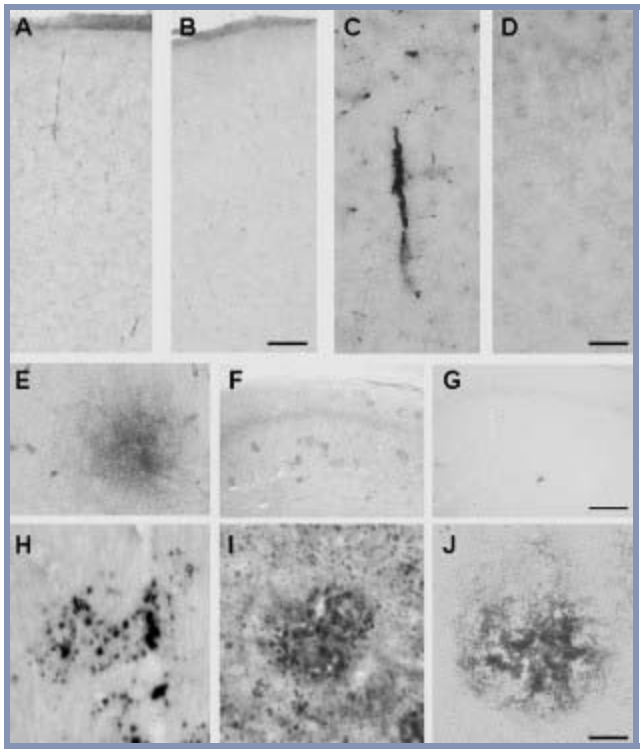
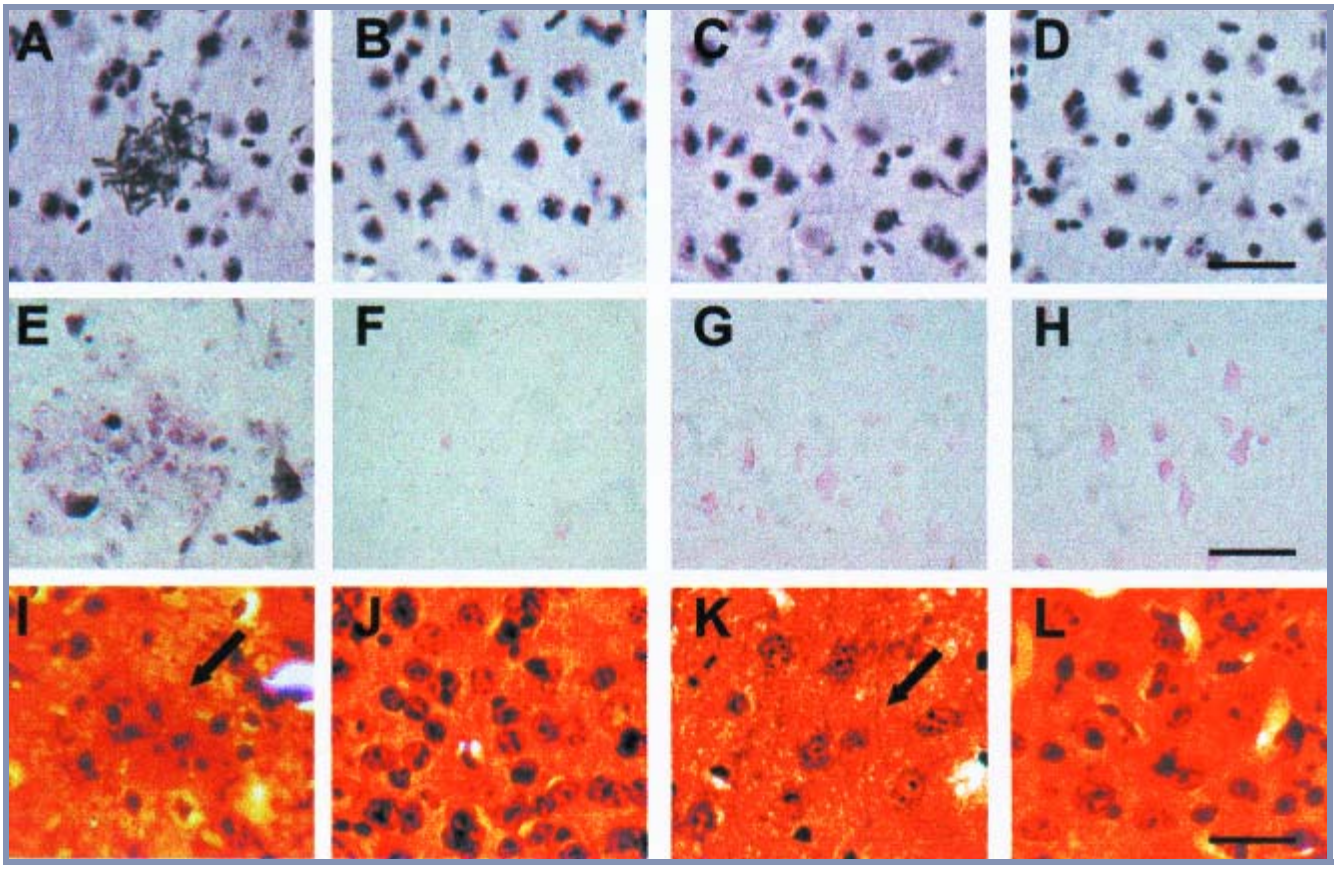


Figure 8. Amyloid plaques in AD11 mice and AD human brains. APP cerebrovascular deposition is observed as early as 2-months of age in AD11 mice **(A)** but not in control mice **(B)**, with an antibody recognizing the carboxyl terminus of APP. β-amyloid deposition in the wall of cerebral vasculature is observed starting from 6-months of age in AD11 mice **(C)** and not in age-matched controls **(D)**, with mAb 4G8 (recognising aminoacids 17-24 of β-amyloid). Extracellular APP deposits are observed cerebral cortex **(E)** of 6 month-old AD11 mice. At this age, the antibody against the NH_2-terminus of Aβ labells clusters of cells in the hippocampus **(H)**. In 15 month-old AD11 mice, the antibody against b-amyloid reveals extracellular deposits of β-amyloid in the hippocampus **(F)**. No staining is observed in age matched control mice **(G)** In panel I an enlargement of a β-amyloid plaque in the cortex **(I)** is shown. For comparison, one plaque from AD human brain is shown in panel J. Scale bars in A,B , F,G 200 µm; in E, H-J = 50 µm.

Figure 9. Amyloid and neurofibrillary pathology revealed by silver impregnation methods. Bielschowsky Silver staining shows the presence of extracellular deposits in 15 months old AD11 mice **(A)** The pattern of staining in 2- and 6-months old AD11 mice and in 15-months control mice is shown respectively in **B,C** and **D**. Gallyas silver impregnation staining reveals intracellular neurofibrillary deposition and extracellular deposits in 15 month old AD11 mice **(E)**. Plaques are surrounded by black stained cells. No staining is detectable in 2- and 6-month old AD11 mice **(F,G)** and in control mice **(H)**. Amyloid compact plaques are observed in 15 month-old AD11 mice using the Campbell-Switzer staining, **(I)** but are virtually absent in 2-months old mice **(J)**. **(K)** At 6 months of age, AD11 mice show dystrophic neurites (arrows) and black intracellular deposition with the Campbell-Switzer staining. No plaques or dystrophic neurites are observed in 15-month old control mice **(L)**. Scale bars in A-B, I-L = 25 µm, in E-H = 50 µm.



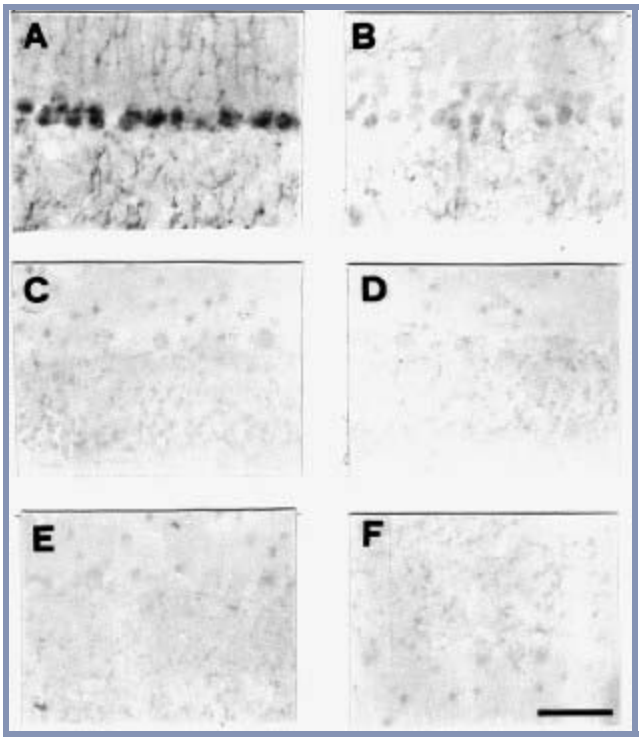


Figure 10. Absence of overt neurodegenerative markers in the cerebellum of AD11 mice. Tau-positive Purkinje cells are found in AD11 mice **(A)**. No labelling is present in age-matched control mice **(B)** or AD11 sections incubated with anti-APP **(C,D)** or anti-tangle antibodies **(E,F)**. Panel **C, E,** AD11 mice. Panel **D,F,** control mice. Scale bar = 75 µm.

Absence of immunolabelling for APP and tangles in the cerebellum of AD11 mice.

The cerebellum is a brain region relatively spared in Alzheimer's disease [50] Therefore the cerebellum of 15-months old AD11 mice was analysed with antibodies against hyperphosphorylated tau (mAb AT8), neurofibrillary tangles (NFT200) and against APP. Immunolabelling with mAb AT8 revealed that Purkinje cells of AD11 mice (Fig. 10A), but not of control mice (Fig. 10B), express the phosphorylation dependent AT8 epitope of tau. No labelling was observed using anti-APP (Fig. 10C,D) or anti-tangles (Fig. 10E,F) antibodies, both in AD11 and in control mice.

Western blot analysis of tau and amyloid proteins

The progressive increase in the amount of phosphorylated tau in the brain of AD11 mice was confirmed by Western blot analysis. Brain extracts were normalised for total protein content with anti-tubulin antibodies (Fig. 11A). Western blotting with the phosphorylation-dependent PHF-1 (data not shown) and AT-8 (Fig. 11B) antibodies showed the appearance of phosphorylated tau in 2 month-old AD11 mice which increased to a plateau from 6 months of age onwards. Western blot with anti-APP showed that an increased amount of APP could be detected starting from 6 months of age, corresponding to an increase of the immunoreactive

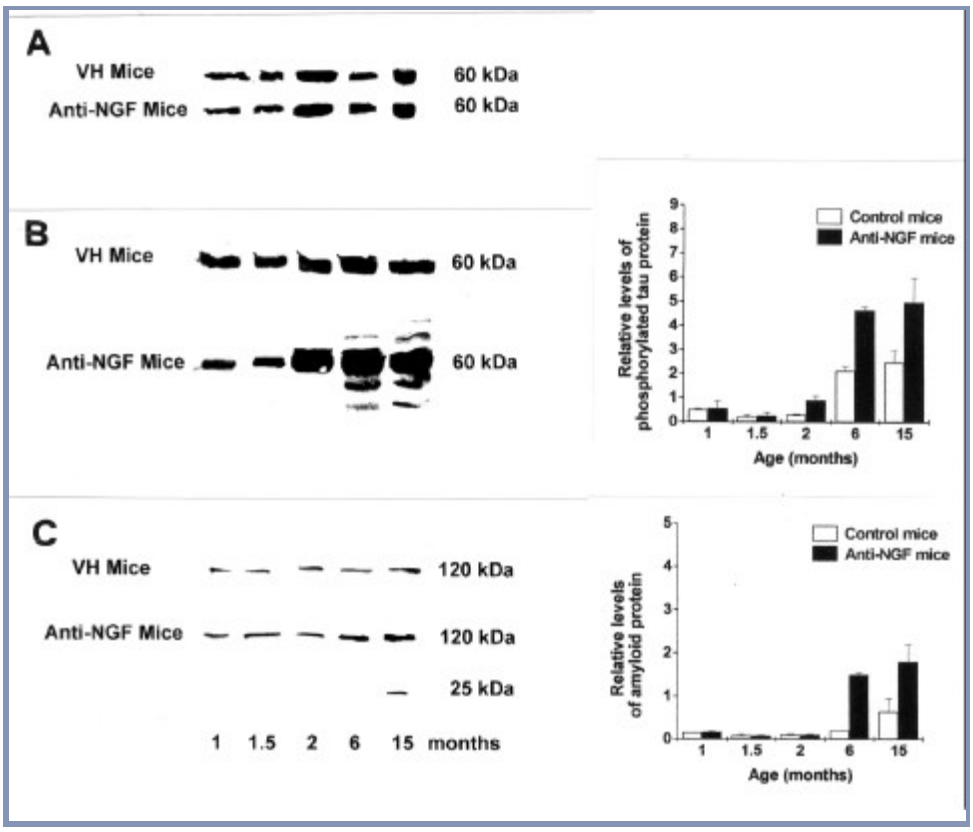


Figure 11.
Western analysis of brain extracts from AD11 and control mice. Blots were probed with mAbs anti-tubulin, (YOL1; **A**), anti-phosphotau (AT8; **B**) and anti-APP **(C)**. Blots are representative of 3 different experiments in which at least 3 control and 3 AD11 mice for each age were used. In each panel upper and lower rows represent blots from extracts derived from control and AD11 mice, respectively. Graphs report quantitative determinations of the intensities of relevant bands. Mean ± S.E.M is shown.

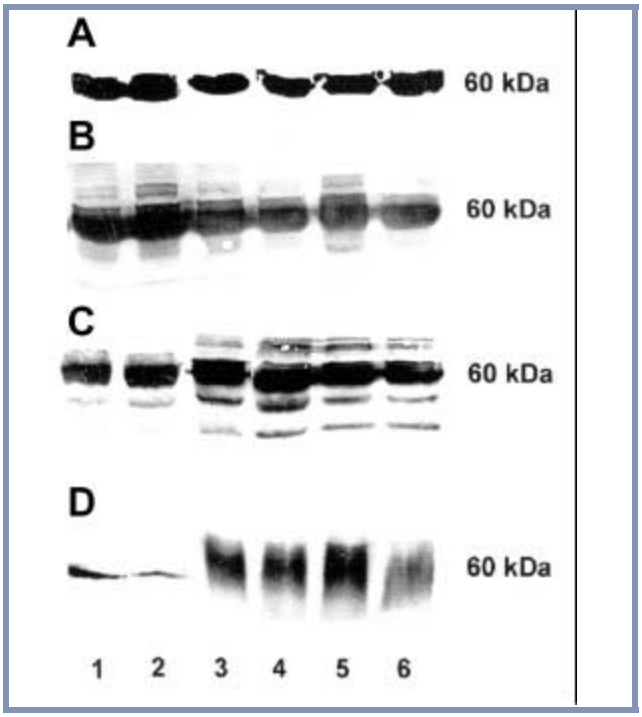


Figure 12. Phosphotau solubility in the brain of AD11 transgenic mice. Insoluble phosphotau protein, detected by mAb AT8, accumulates in the brain of 15 month-old mice. Protein amount was checked using anti-tubulin YOL1 antibodies **(A)**. Tissues were sequentially extracted with RAB Hi-salt **(B)**, RIPA buffer **(C)** and 70% FA **(D)**. RAB-insoluble tau, represented by the RIPA and FA fractions, accumulates in the brain of AD11 mice but not in control mice. Lanes 1-2 refer to controls; lanes 3-5 to AD11 mice.

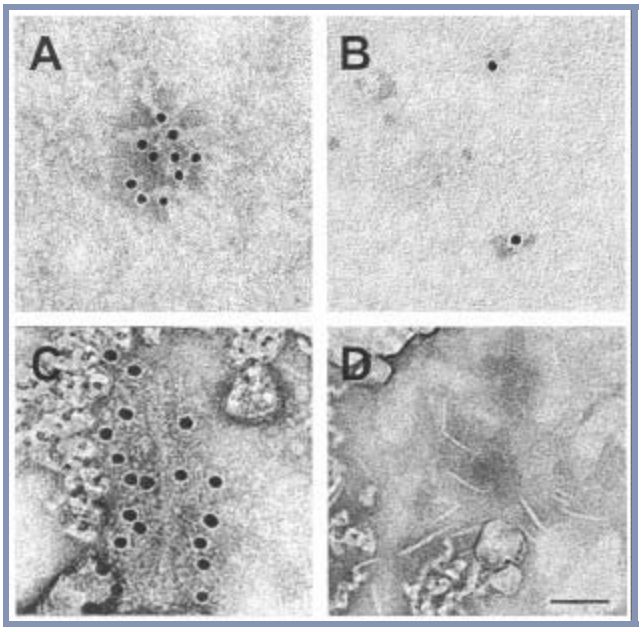


Figure 13. Immunoelectron microscopy of dispersed PHF preparations in **(A)** 15 months old AD11 mice and **(C)** human AD. No immuno-decoration is observed in extracts from **(B)** age-matched control mice and **(D)** human non demented brains. **(E)** The graph shows the progression of neuronal loss in AD11 mice as measured by stereological counts in layer V of cerebral primary visual cortex.

120 kDa band (Fig. 11C). Western analysis showed also a differential processing of APP in extracts from 15 month-old AD11 mice, with the appearance of a new 25 kDa immunoreactive band (Fig. 11C).

Tau solubility in aged AD11

Tau aggregates, containing the insoluble form of the protein, constitute a hallmark of AD and other tauopathies[51]. Previous evidence suggested the presence of insoluble tau in total brain homogenates of aged AD11 mice [31]. Here we performed a differential extraction procedure [43], similar to that used to solubilize tau from PHFs in AD brains, to determine the presence and the amount of insoluble tau in aged (15 month-old) AD11 mice. Brain samples were sequentially extracted with RAB Hi-salt buffer, RIPA buffer and 70% FA. In transgenic control mice, tau was largely RAB-soluble, some tau was found in the RIPA-fraction, while only a small amount was found in the FA-soluble fractions (Fig. 12). In AD11 mice, the RAB-soluble fraction was somewhat reduced (Fig 12 B, lanes 3 to 6 versus lanes 1 and 2), while a large amount of RAB-insoluble tau, represented by the RIPA- and FA-fractions, accumulated (Fig 12 C and D). This firmly establishes the presence of insoluble forms of tau in the brain of aged AD11 mice. The nature of these tau aggregates remains to be determined, but they could be related tau aggregation in PHF-like structures, as described below.

Immunoelectron microscopy reveals the presence of PHFs in brain of aged AD11 mice

Dispersed PHF preparations were made by sarcosyl extraction from entorhinal cortex of 2 human subjects and 12 mice (six 15 months old AD11 mice and six age-matched control mice). Immuno-electron microscopy with mAb AT8 and gold particles showed that all preparations from AD11 mice (Fig. 13A) and from AD human brains (Fig. 13C) contained protofilaments of PHFs. The filament morphology were indistinguishable between human AD and AD11 mice, except the average length of PHFs, that was shorter in AD11 mice. The diameter of filaments was measured being between 1.9 and 2 nm both in human AD and AD11 mice. The antibody AT8 labeled tau associated to fuzzy coat of PHFs, as reported previously [52]. Preparations made from age-matched control mice (Fig. 13B) and human non demented brains (Fig. 13D) contained neither filaments nor AT8-positive PHFs.

Co-expression of transgenic antibody chains and markers of neurodegeneration

The localisation of VH and VK transgenic antibody chains with markers of neurodegeneration such as hyperphosphorylated tau and β-amyloid was investigated

Ebixa® – the name

Product information (abbreviated from the SmPC) Name: Ebixa®. **Active substance:** Memantine. **Indication:** Treatment of patients with moderately severe to severe Alzheimer's disease. **Contraindications:** Hypersensitivity to active substance or any of the excipients. **Special warnings and precautions:** Not recommended for patients with severe renal impairment. Caution is recommended with patients suffering from epilepsy. Clinical data are limited on patients with myocardial infarction, congestive heart failure and uncontrolled hypertension and patients with these conditions should be closely supervised. **Interactions:**Concomitant use of amantadine, ketamine or dextromethorphan should be avoided. Effects of L-dopa, dopaminergic agonists and anticholinergics may be enhanced. Effects of barbiturates and neuroleptics may be reduced. Effect of dantrolene and baclofen may be modified. Plasma levels of cimetidine, ranitidine, procainamide, quinidine and nicotine may be increased. Urinary pH increase may elevate plasma levels of memantine. **Common adverse reactions:** Hallucinations, confusion, dizziness, headache and tiredness (none above 2%). Uncommon adverse reactions (0,1 – 1%): Anxiety, increased muscle tone, vomiting, cystitis and increased libido.

to remember

PostScript Picture
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The first and only drug in a new class – effective even in severe stages of Alzheimer's disease[1,2]

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Posology: Maintenance dose is 20 mg, (10 mg twice daily) taken with or without food. Treatment starts with 5 mg in the morning for a week; the 2nd week 5 mg twice daily; the 3rd week 10 mg in the morning and 5 mg in the afternoon and from 4th week 10 mg twice daily. Reduce dose to 5 mg twice daily in patients with moderate renal impairment. **Overdose:** Symptomatic treatment. Elimination: Mainly in unchanged form via the kidneys. **Administration:** Orally as tablets (10 mg) or solution (10 mg/g). **Marketing authorisation:** H. Lundbeck A/S, 9 Ottiliavej, DK-2500 Valby, Denmark. **References: 1.** Winblad B, Poritis N. Memantine In Severe Dementia: Results Of The M-Best Study (Benefit And Efficacy In Severely Demented Patients During Treatment With Memantine). Int J Geriat Psychiatry 1999;14:135-146 **2.** Reisberg B, Windscheif U, Ferris SH, Stoeffler A, Moebius H-J, and the Memantine Study Group. Memantine in moderately severe to severe Alzheimer's disease: results of a 6-month multicenter randomized controlled trial. Neurobiology of Aging 2000; 21 (1S): S1275.

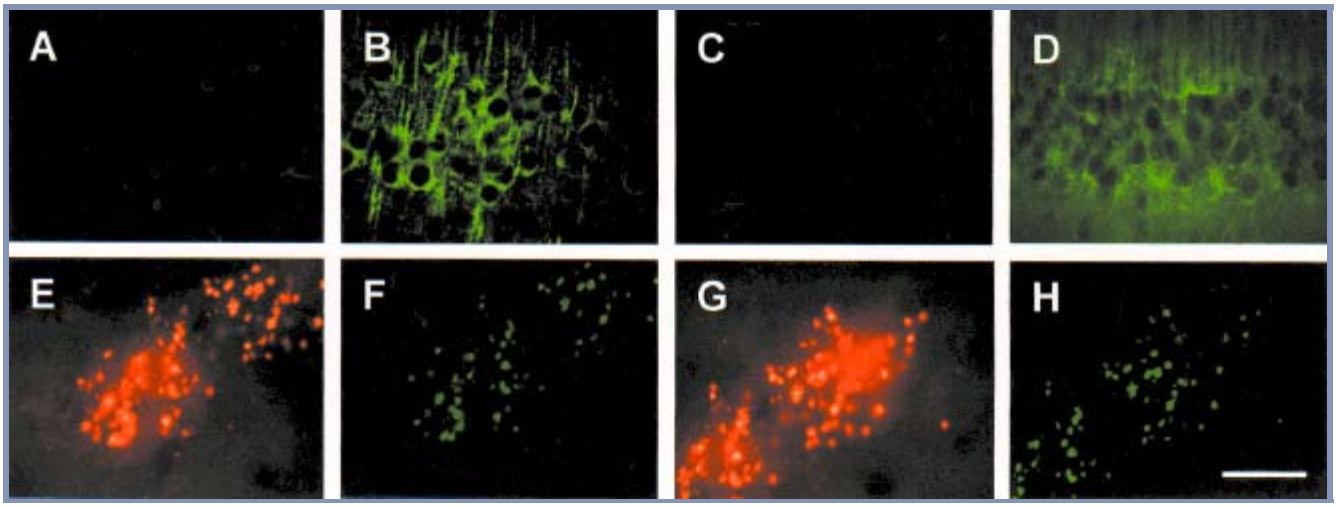

Figure 14. Co-expression of transgenic antibody chains and APP, phosphotau or b-amyloid. Double immunohistochemistry against phosphotau **(A, C)** and VH **(B)** or VK **(D)** do not show co-localisation of phosphotau and transgenic antibody chains in 2 month-old AD11 mice. At this age, phosphotau is not present in hippocampal neurons **(A,C)**, while a high expression of both VH **(B)** and VK **(D)** chains is observed.
At 15 months of age, cells surrounding β-amyloid plaques (**E,G**; yellow labelled cells) are positive also for VH **(F)** and VK **(H)** chains. No labelling for VH and VK chains is shown in the extracellular deposits. Scale bars in A-D = 200µm, E-H = 75 µm.

by double immunohistochemistry at 2 and 15 months of age, respectively. In the hippocampus, no expression for hyperphosphorylated tau was detected at 2 months of age (Fig. 14A,C), while most pyramidal cells expressed VH (Fig. 14B) or VK chains (Fig. 14D). At 15 months of age, AD11 mice displayed a high number of β-amyloid plaques encircled by cells (Fig. 14E,G). Only the cells surrounding the plaques expressed both transgenic chains and β-amyloid, while the extracellular amyloid material did not appear to contain VH or VK chains (Fig. 14F and 14H).

Discussion

We have recently produced transgenic mice expressing the recombinant form of the AD11 neutralising antibody αD11 [30], which develop with age a neuropathology strikingly similar in many aspects to that of AD [31,32]. Histological and biochemical analysis of aged AD11 transgenic mice revealed cholinergic deficits, neuronal loss and degeneration of neurons and dendrites in the cerebral cortex and hippocampus, resulting in the presence of neurofibrillary tangles, extracellular deposits of APP immunoreactive material and β-amyloid plaques [31,32]. The neurodegeneration appeared to be strictly related to behavioural [30, 31] and cortical synaptic plasticity deficits [33].

A retrospective staging of AD has shown that the disease is characterised by a well-defined temporal sequence of morphological changes that correlate with the clinical course of the disease [53]. Thus, it is of interest to find a link between AD staging in human brain and the time course of the neurodegenerative phenotype observed in AD11 mice. This would be crucial for the development of an experimental system for screening potential therapeutic agents that can prevent neurodegeneration at early stages.

As a further step in this direction, in this paper we investigated the spatio-temporal pattern of progression of a wide set of phenotypic markers for degeneration, including the accumulation of β-amyloid immunoreactive material in extracellular deposits.

Moreover, to carry out this study, a second independent transgenic line was used, for all the phenotypic analyses performed. The pathological characteristics of AD11 mice are remarkably consistent among the two different lines of AD11 transgenic mice analysed (families 1 and 2 [30]). This extends and further confirms the results of our previous report [31], in which only one family (family 1) was analysed.

Different control experiments were performed. First, we verified that, as demonstrated before by Piccioli *et al.* [54] for another line of transgenic mice expressing "neuroantibodies", no anti-human IgG response was elicited in AD11 transgenic mice (data not shown). Moreover, we used transgenic mice expressing only the heavy chain of aD11 antibody as a control. It has to be underlined that this VH control mouse is a very stringent control, since it allows to verify the possible "non specific" contribition of the potentially toxic heavy chain to the neurodegenerative phenotype. A further control for the regional specificity of the observed effects of NGF deprivation was performed by comparing results on forebrain sections with results observed on cerebellum and spinal cord. We show that at 15 months of age, when all the panel of pathological characteristics are present in the forebrain, Purkinje cells are the only phosphotau-positive neurons in the cerebellum of AD11 transgenic mice, while

no sign of phosphotau accumulation was observed in the spinal cord. Moreover, no anti-tangle or anti-APP immunopositivity was detected in both cerebellum and spinal cord in spite of the fact that transgenic antibodies are expressed in this areas[30]. Therefore, the spatial pattern of neurodegeneration observed in AD11 mice correlates well with that observed in human AD, where the cerebellum and spinal cord are relatively spared [50].

Immunoglobulin light chain and heavy chain have been also associated to organ-compromising amyloid-like deposits in tissues, giving rise to amyloidotic diseases [55,56]. To exclude that in AD11 mice APP or β-amyloid plaques and intracellular tau hyperphosphorylation could be related to an abnormal deposition and aggregation of the transgenic chains, we performed double immunohistochemistry in the hippocampus, one of the brain regions showing a high expression of both light and heavy chains [30], as well as of amyloid deposits. We showed that neither the VK nor VH chains are co-localized with the extracellular component amyloid plaques and that in hippocampal neurons the expression of phosphotau does not coincide with that of transgenic antibody chains. Thus the neurodegenerative phenotype appears not to be related to gammopathies or amyloidotic-light chain diseases. As a further demonstration of the fact that neurodegeneration in AD11 mice is not due to the expression of antibody chains per se, but to the expression of anti-NGF antibodies, we demonstrated that the phenotype can be reverted by the delivery of NGF to the CNS of AD11 mice through an olfactory route[57].

In order to investigate the temporal progression of the neurodegeneration, we selected a set of phenotypic markers chosen to characterise the state of the neuronal cytoskeleton, the cholinergic phenotype and the processing of proteins linked to AD, such as tau, APP and its proteolytic product β-amyloid, as well as histochemical markers (summarised in Table 1).

Table 1.

		AGE (MONTHS)								
PHENOTYPIC MARKERS	**BRAIN AREAS**	1	1.5	2	4	6	8	10	12	15
ChAT reduction	Basal forebrain	–	–	+	+	++	++	++	++	++
AT180	entorhinal cortex	–	–	–	–	+	+	+	++	++
	parietal cortex	–	–	–	–	+	+	+	++	++
	occipital cortex	–	–	–	–	+	+	+	++	++
	hippocampus	–	–	–	–	–	–	–	–	–
AT270	entorhinal cortex	–	–	–	–	+	+	+	++	++
	parietal cortex	–	–	–	–	+	+	+	++	++
	occipital cortex	–	–	–	–	+	+	+	++	++
	hippocampus	–	–	–	–	–	–	–	+	+
AT8	entorhinal cortex	–	–	+	+	++	++	++	+++	+++
	parietal cortex	–	–	–	+	+	+	++	+++	+++
	occipital cortex	–	–	–	+	+	+	++	++	+++
	hippocampus	–	–	–	+	+	+	++	++	+++
Dystrophic neurites [a]		–	–	–	–	+	+	++	++	+++
Neurofibrillary tangles		–	–	–	–	–	–	–	+	+++
PHFs		–	–	ND	ND	ND	ND	ND	ND	+++
Insoluble tau		–	–	ND	ND	ND	ND	ND	ND	+++
MAP-2 altered distribution		–	–	+	+	++	++	++	+++	+++
Cerebrovascular APP		–	–	+	+	+	+	+	+	+
Extracellular APP deposition		–	–	–	–	+	+	+	+	+
Cerebrovascular Aβ		–	–	–	–	+	+	+	+	+
Intracellular Aβ		–	–	–	–	+	+	+	+	+
β-amyloid plaques		–	–	–	–	–	–	–	–	+
Extracellular deposits[b]		ND	ND	–	ND	–	ND	ND	ND	+
Neuronal loss		–	–	–	–	–	+	+	++	++
DNA fragmentation		–	–	–	–	–	–	–	–	+

The symbol + indicates a qualitative measure of each phenotypic marker. Where appropriate, quantification has been reported in the text, figures or in Capsoni et al. (2000a). ND, not done.
The Table reports only those phenotypic markers that show a significant difference with respect to age-matched control littermates.
[a] Revealed by silver impregnation and immunohistochemistry with antibodies raised against hyperphosphorylated tau and tangles.
[b] Revealed by silver impregnation methods.

Here we show that none of the tested phenotypic markers of degeneration is altered at 1.5 month of age, when the recombinant AD11 antibodies are already clearly detectable[31]. The first signs of phenotypic alterations appear at 2 months of age, namely: i) 50 % reduction of ChAT-positive neurones, ii) hyperphosphorylated tau in the entorhinal cortex (revealed with mAb AT8), iii) reduction of the number of cortical dendrites immunostained with MAP-2 and redistribution of the staining pattern. From 2 months onwards, each of the markers analysed shows a well defined and distinct temporal progression, and only in 15 months old mice the full complement of markers is significantly altered (see Table 1).

Amyloid deposits, as revealed using the anti-APP antibody, are first seen at 6 months of age, and also in this case the amyloid burden is initially higher in the entorhinal cortex than elsewhere. In this paper we report also the presence of β-amyloid deposits in 15-months old mice, as revealed using the anti- β-amyloid antibody 4G8, which recognizes residues 17-24 of the β-amyloid protein. Neurofibrillary tangles, as revealed by an anti-tangle monoclonal antibody and by the presence of protofilaments of PHF by immunoelectron microscopy, are only found at 15 months of age, although silver impregnation reveals tangle-like structures also at 6 months.

The AD-like alterations observed in aged AD11 mice are shown, here, to be very similar to those observed in human AD brains. A parallel comparison with human AD brain sections revealed that the flame-like shape of neurones stained with the anti tangle NFT200 antibody is highly reminiscent of human neurofibrillary tangles.

Also, we show here that extracellular deposits immunoreactive with antibodies to β amyloid are found in brains of aged AD11 mice [32] , albeit at a somewhat lower frequency than the APP-immunoreactive deposits. This is remarkable, since in rodents the formation of amyloid plaques has not been previously reported, unless mutated human APP was overexpressed [58]. For this reason, it can be concluded that AD11 mice could provide an experimental animal model for events leading to the sporadic, more frequent form of AD.

The data presented here provide compelling evidence to support the hypothesis that the cholinergic impairment and an impairment of cytoskeletal function revealed by an abnormal MAP-2 subcellular distribution and tau phoshorylation are critically involved in the early stages of the development of an AD-like neurodegeneration induced by interfering with NGF activity.

It is noteworthy that in 2-months old mice, abnormal tau immunoreactivity is first localised in neurones of the entorhinal cortex. This brain area corresponds to the human medial temporal lobe region that is affected early in the disease process, as described with both histopathological [53,59-61] and brain imaging techniques [62]. Similarly to what is observed in AD patients, also in AD11 mice the neurodegenerative process spreads from the entorhinal cortex to other neocortical areas, affecting mainly neocortical areas and the hippocampal formation (mostly subiculum). Regions such the neostriatum (data not shown) and the cerebellum are poorly affected by the neurodegeneration in AD11 mice.

In the cortex of AD patients, a high degree of vulnerability is displayed in pyramidal-like cells of layers II, III and V [63-66]. Interestingly, in 2 and 6 month-old AD11 transgenic mice tau hyperphosphorylation starts to be detected in cortical layers II and III and then, with ageing, it spreads to the other layers. Thus, the spatio-temporal progression of affected cortical neurones in AD11 transgenic mice, including laminar organisation, is similar to that observed in AD human brains and suggests the involvement of specific cortico-cortical connections[59, 67]. In relation to this aspect, a particular aspect is shown in AD11 mice, where a high degree of neuronal loss is shown at the level of the primary visual cortex. Patients affected by a variant of Alzheimer's disease may show anomalies of higher cortical visual function[68], hypometabolism[69] and histological anormalities of pyramidal neurons in layer V of the visual cortex [70, 71]. Interestingly, in this paper we report a decrease of the same neuronal population in AD11 mice.

Also of note is the fact that the first signs of abnormal APP and β-amyloid deposition in AD11 mice are vascular and perivascular deposition, which become evident much earlier than the extracellular APP and β-amyloid plaques. It is noteworthy that the selective destruction of the cholinergic nucleus basalis in the rabbit by a p75 immunotoxin leads to the deposition of β-amyloid in and around cerebral vessels [72].

The panel of antibodies against different epitopes of phosphorylated tau that have been used in this study, have been previously and widely used to detect paired helical filaments (PHFs) and tangles [52, 73, 74] . Our experiments reveal that different antibodies reveal a distinct spatio-temporal progression of expression as well as a distinct subcellular distribution in AD11 mice. We do not have, at present, an explanation for this finding, even if they may relate to the sequential changes in tau immunoreactivity during the development of AD paired helical filaments and tangles[52,74]. It remains to be seen whether in AD11 mice also other types of abnormal post-translational processing of tau, such as glycosylation [75] or proteolysis [76-78] are occurring. The results obtained are in line with what reported before by Goedert *et al.* [73], showing a decrease in expression of AT8, AT180 and AT270 epitopes in rat brains starting from 2 months of age. However, since that analysis was performed until 4 months of age, it is impossible to compare the results obtained in older AD11 mice with previous reports.

The presence of neurofibrillary tangles was revealed by three different methods, namely by silver impregnation methods widely used to monitor AD neurofibrillary

pathology, immunohistochemistry using an antibody against neurofilaments previously shown to react specifically with neurofibrillary tangles in human AD brains [49,79] and immunoelectron microscopy on L-sarcosine fractions of brain extracts. Consistently with the finding of tau containing tangles, in aged AD11 mice, a substantial fraction of tau protein is found in an insoluble form, that can be solubilized only with formic acid, a solvent that has been used to extract tau from AD PHF [80].

In conclusion, AD11 mice recreate many aspects of abnormalities that are also seen in brains of AD patients. The phenotype of these mice leads to suggest that NGF is not only a critical component of the molecular mechanism of AD neurodegeneration [31,32,57], but also that the timing of NGF deprivation is crucial for the extent of the neurodegeneration. Indeed, in a previous paper [30] we pointed out that the differences observed between AD11 mice and adult *ngf* +/- mice [81] could be due mainly to a critical period of sensitivity of basal forebrain cholinergic neurones to NGF deprivation. A negative priming model [82], whereby the absence of NGF during the early postnatal development would lead to acquisition of NGF independence from NGF itself during adulthood can be suggested by comparing our results [31,32], (this paper) with those reported for *ngf* -/- and *ngf* +/- mice [81,83] Thus, the onset of the neurodegeneration would be related not only to the amount of free NGF available in transgenic mice but also to the time of mouse life in which NGF deprivation begins.

In any case, the phenotype displayed by AD11 mice provides compelling evidence that NGF plays a fundamental role in the development (and henceforth in the prevention) of Alzheimer's neurodegeneration, in keeping with recent results showing that retrograde NGF signalling by cholinergic neurons is impaired in a mouse model of Down's syndrome related to AD[17]. In human AD, recent evidence demonstrating alterations in NGF processing [12] and a reduced expression of TrkA [22] also point to a compromised and hypofunctional NGF signalling in cholinergic neurons. Our results provide additional evidence for the functional link between neurotrophin signalling, cholinergic mechanisms and APP processing [84].

Since our temporal analysis has revealed that the earliest events observed are a cholinergic deficit and cytoskeletal abnormalities, this transgenic model will allow studying the molecular links between NGF reduction, cholinergic activity and the post-translational regulation of cytoskeletal proteins.

Further studies are needed to clarify the mechanisms whereby NGF deprivation determines an AD-like neurodegeneration from the mechanistic point of view. We cannot exclude that neurodegeneration may be due not only to the NGF neutralisation per se but also to the particular mode of this neutralisation, and this may have deep implications for human AD.

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CGI-94 Controls Neuronal Survival

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Abstract

Active cell death ('apoptosis' or 'programmed cell death') is essential in the development and homeostasis of multicellular organisms and abnormal inhibition of apoptosis is an indicator of cancer and autoimmune diseases, whereas excessive cell death is implicated in neurodegenerative disorders such as Alzheimer's disease (AD). Here we show that Smac/Diablo (second mitochondria-derived activator of caspases) co-localizes with CGI-94 (comparative gene identification-94) in the cell. CGI-94 itself contains an utp11-motif and is probably involved in ribosome biogenesis and degenerative processes of AD. These findings point to a pivotal role of CGI-94 in the control of cellular survival.

Keywords: cell death, differentiation, nerve growth factor, neurodegeneration

Introduction

Apoptosis is an active form of cell death with an important role in development and homeostasis in multicellular organisms. Cell death by apoptosis comprises a sequence of events leading to the activation of caspases, which execute the fragmentation of the cellular protein and DNA leading to disintegration of the cell. This physiological neuronal apoptosis allows the nervous system to eliminate excess neurons. In addition, apoptotic cell death occurs in a variety of neuronal degeneration such as Alzheimer's disease (AD)[1,2]. The disease is characterized by the presence of neuritic amyloid plaques, cerebrovascular amyloidosis and neurofibrillary tangles[3].

Recently, we found an alteration in expression of the new multifunctional protein CGI-94 (comparative gene identification-94) in the hippocampus of early stage AD brain[4]. In addition, we could demonstrate that - even in the presence of nerve growth factor (NGF) - CGI-94-positive neurons undergo cell death[5]. Moreover, we investigated the cellular function of CGI-94 by applying the two-hybrid-system and found that CGI-94 is interacting with the pro-apoptotic protein Smac/Diablo (second mitochondria-derived activator of caspases)[5].

To further explore the cause of neuronal degeneration in AD we describe in the present study the functional role of CGI-94 by analyzing the possible pathophysiological outcome of intracellular CGI-94-expression. Disclosing the possible mechanism by which CGI-94 is mediating neuronal cell death we have sub-cloned the open reading frame of CGI-94 in-frame with the green fluorescent protein (GFP) to study the subcellular localization of CGI-94-GFP and Smac-DsRed1 fusion proteins by using fluorescent light microscopy as it has been described recently as a novel visual classification approach[6]. We co-expressed CGI-94-GFP and Smac-DsRed1 proteins in CHO (Chinese hamster ovary) cells and demonstrate for the first time that CGI-94 and Smac co-localize in the cell.

RNA intereference (RNAi) is the process of sequence-specific, post-transcriptional gene silencing in animals and plants, initiated by double-stranded RNA (dsRNA)[7]. This method of assessing gene function involves the introduction of small interfering RNA (siRNA) duplexes into cells. These short duplexes are 21-23 nucleotides in length and are designed to specifically target and silence a particular messenger RNA (mRNA) of interest.

Here we show that siRNA duplexes specifically suppress expression of endogenous CGI-94 gene in rat neuronal B104 cells which results in neuronal cell death.

A large ribonucleoprotein (RNP) complex purified from *Saccharomyces cerevisiae* contains a) the U3 small nucleolar RNA (snoRNA) - which is ubiquitous in eukaryotes and is required for nucleolar processing of pre-18S ribosomal RNA - and b) 28 proteins including 17 called Utp1-17. The Utp proteins are nucleolar and specifically associated with the U3 snoRNA. Depletion of the Utp proteins impedes production of the 18S rRNA. The Utp proteins are therefore required for biogenesis of the small ribosomal subunit RNA, as are all of the other known U3 snoRNP-specific components[8].

In the present study we identified human/rat CGI-94 as the potential homologous protein of yeast Utp11 enlightening our observation of CGI-94-specific siRNA-mediated cell death.

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Methods

Reagents

Unless indicated, all reagents used for biochemical methods were purchased from Sigma-Aldrich (Tokyo, Japan).

Sub-cloning

Human *CGI-94* cDNA was obtained as described previously[4]. A CGI-94-GFP expression construct was generated by inserting human *CGI-94* cDNA in-frame with the green fluorescent protein (GFP) of pcDNA3.1CT-GFP-TOPO® (Invitrogen, Tokyo, Japan) at the C-terminus of CGI-94 (CGI-94-GFP).

The open-reading-frame of human *Smac* (human Smac (239 aa) has been cloned from human embryonic kidney cells (HEK293-cell line) according to its sequence BAB14994 using specific primers: sense: 5'-atg-gcg-gct-ctg-aag-agt-tgg-ctg-tcg-cgc-agc-gta-3'; anti: 5'-tca atc ctc acg cag gta ggc ctc ctg ctc cga-3') *Smac* has been cloned in-frame with a *DsRed1* expression vector (Clontech) as *Smac-DsRed1* (DsRed1 is at the C-terminus of Smac) using NheI and Hind III restriction-enzyme-cloning sites.

Cell culture

CHO and rat neuronal B104 cells were propagated in Dulbecco's Modified Eagle Medium (D-MEM) containing 10 % fetal calf serum (FCS; Gibco BRL, Grand Island, NY, USA) at 37 °C in humidified 5% CO_2/95% air.

Transfection

CHO cells were transiently transfected with CGI-94-GFP and Smac-DsRed1 expression vectors using SuperFector transfection reagent (according to the manufacturer's protocol; B-Bridge, San Jose, CA, USA) and maintained in D-MEM medium containing 10 % FCS (Gibco) at 37°C in humidified 5% CO_2/95% air[4,7]. Additionally, transfection efficiency (about 60-70 %) was always confirmed by co-transfection of GFP reporter transcripts (with or without CGI-94) with p53-/PKC-DsRed1 (Clontech, Tokyo, Japan) transcripts as reported recently[7]. Cell survival of CGI-94-GFP/Smac-DsRed1 positive cells was assessed after 48 hrs post transfection by fluorescence microscopy (Olympus IX70) and measured using the CellTiter 96® AQ_{ueous} Assay (Promega)[5].

siRNA survival assay

B104 cells were regularly passaged to maintain exponential growth. 24 h before transfection at 50-80% confluency, cells were trypsinized and diluted 1:5 with fresh medium without antibiotics (1-3 x 10^5 cells/ml) and transferred into 24-well plates (500 µl/well). Transfection of 0.5 µg siRNA-duplex/well was carried out with Oligofectamine (Invitrogene) as described by the manufacturer for adherent cells. Transfection efficiencies were determined by fluorescence microscopy after co-transfection of 1 µg GFP-expression vector and 0.2 µg siRNA-duplex/well as described recently[4,7,9]. Specific silencing of target genes was confirmed by at least three independent experiments. Cell survival was measured by CellTiter96® AQ_{ueous} Assay (Promega)[5]. For controls, the single-stranded sense, complementary RNA oligos (= control-1, single-stranded siRNAs) and a duplex with the inverted CGI-94 sequence (= control-2) were used. The siRNA sequence targeting CGI-94 was from position nt44 – nt64 and nt79 – nt99 relative to the start codon. CGI-94 specific 21-nucleotide siRNA-duplex ready for transfection was obtained from Dharmacon Research (Lafayetta, CO, USA) and B-Bridge International, (Tokyo, Japan).

Results

Expression of CGI-94-GFP and Smac-DsRed1 in CHO cells

To investigate the possible pathophysiological function of CGI-94 we co-transfected CHO cells with a CGI-94-GFP- and a Smac-DsRed1-fusion protein. Characterizing the expression of CGI-94-GFP by fluorescence microscopy indicates that CGI-94 is transferred into the nucleus according to its bipartite nuclear localization signal (NLS) motif (Fig, 1E", F",G")[4]. However, as shown in Figure 1A", B", C", it could also be detected in the cytoplasm of the cell. Comparing with Smac-DsRed1-co-transfected cells it seems that CGI-94 is entering mitochondrial compartments of the cell, because Smac is known to enter mitochondria (Fig.1A', B', C') and Figure 1 (A", B", C") clearly shows the co-localization of CGI-94-GFP and Smac-DsRed1.

Survival assay using small interfering RNA (siRNA)

In Figure 2 we demonstrate that 21-nucleotide siRNA duplexes specifically suppress expression of endogenous CGI-94 gene in rat neuronal B104 cells. This specific CGI-94 mRNA suppression results in neuronal cell death. The knock-down phenotype became apparent after 3-5 days.

In addition, sequence motif search at http://www.expasy.ch/ revealed that CGI-94 has an utp11-motif and is probably the human/rat homologous protein of yeast Utp11 (locusID: 51118/UniGene: Hs.111449)[4,8]. Utp11 is part of the small (ribosomal) subunit (SSU) processosome (contains U3 snoRNA).

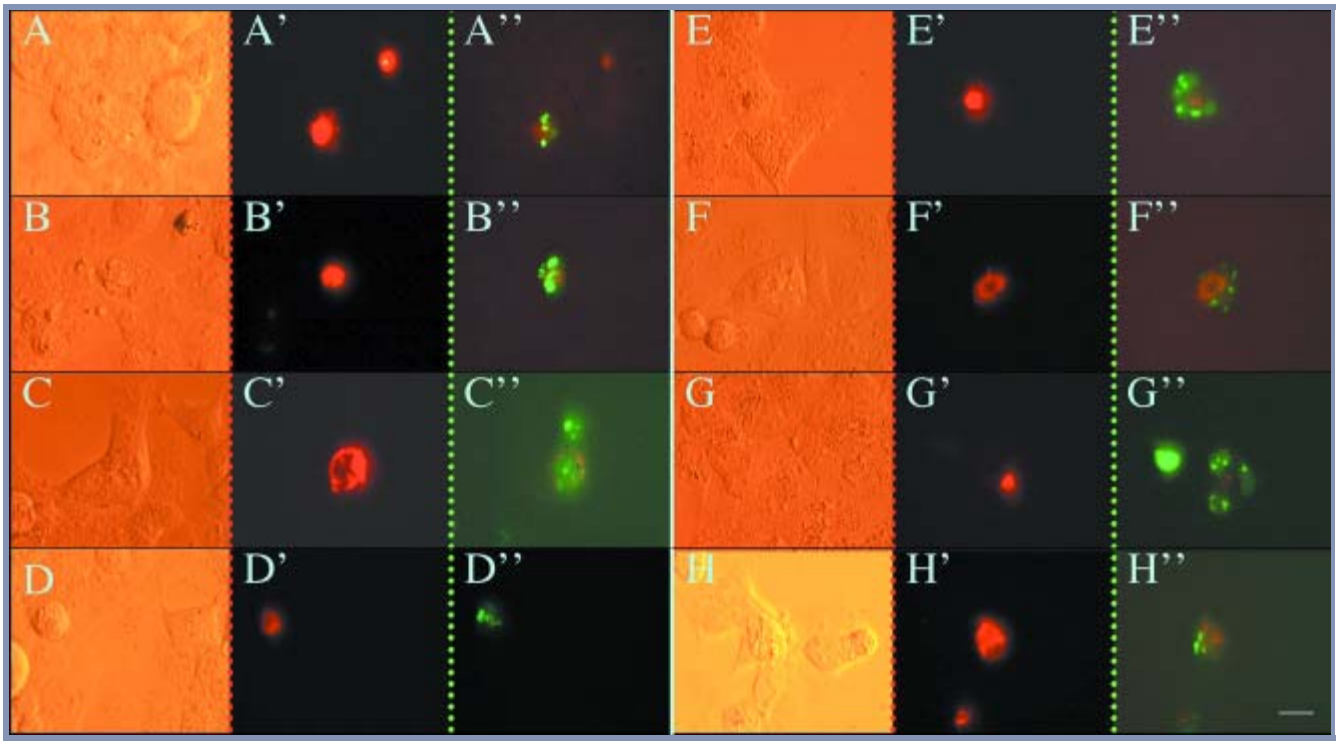

Figure 1. Fluorescence co-localization of CGI-94-GFP and Smac-DsRed1 expression in CHO cells. CHO cells transiently co-transfected with CGI-94-GFP and Smac-DsRed1 were visualized directly by CGI-94-GFP-green or Smac-red fluorescence showing a nuclear CGI-94 expression- and a mitochondrial CGI-94-/Smac-co-expression pattern. The left panels (A-D and E-H) show cells in phase contrast modulation, and the middle and right panels (A'-D', E'-H' and A"-D", E"-H") show cells of the same field under fluorescence. The bar indicates 75 µm.

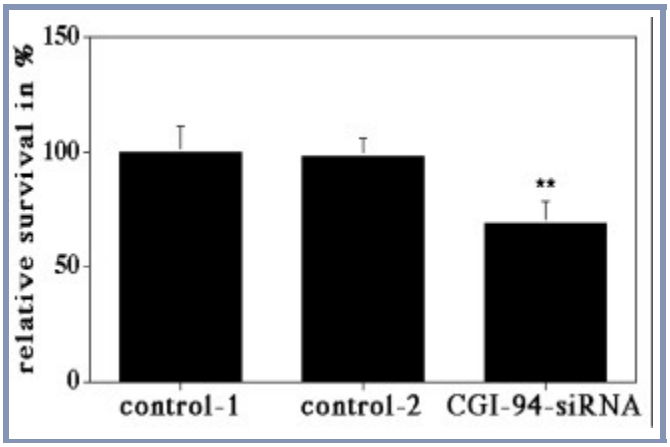

Figure 2. Effect of CGI-94-specific siRNA on survival of B104 cells. ELISA-CellTiter 96® AQ_{ueous} Assay (Promega). Cells were incubated as described in experimental procedures. Data are shown as mean ± SEM of eight independent experiments, each done in duplicate (**P < 0.01, compared to controls, ANOVA).

Discussion

Cell death by apoptosis comprises a sequence of events leading to the activation of cysteine proteases, called caspases, that are synthesized as inactive precursors and that are proteolytically processed to generate active subunits until caspase-3, the final executioner, is activated[1,10]. Caspase activation by p53 occurs through the release of apoptogenic factors from the mitochondria, including cytochrome-c and Smac/Diablo. The release of mitochondrial cytochrome-c can be triggered through p53-induced activation of Bax in a caspase-independent manner. Additionally, many functions of p53 are exerted by its activity as a transcriptional activator of Bax, Noxa and PUMA, which have the ability to induce mitochondrial cytochrome-c release. The mitochondrial pro-apoptotic protein Smac/Diablo has recently been shown to potentiate apoptosis by counteracting the anti-apoptotic function of the inhibitor of apoptosis proteins (IAPs). In response to apoptotic stimuli, Smac is released into the cytosol along with cytochrome-c and promotes caspase activation by binding to IAPs, thereby blocking their function.

During neuronal damage cytochrome-*c* is released from mitochondria into the cytosol and induces ATP-/dATP-dependent formation of a complex of proteins such as Apaf-1 and caspase-9 that results in the proteolytic activation of procaspase-3. Concurrent with cytochrome-c release Smac/Diablo is released from the mitochondria into the cytosol, eliminates the inhibitory effect of all IAPs that have been examined (including XIAP, c-IAP1, c-IAP2 and survivin) and mediates apoptosis via promoting cytochrome-*c*-dependent caspase-3 activation[10-13].

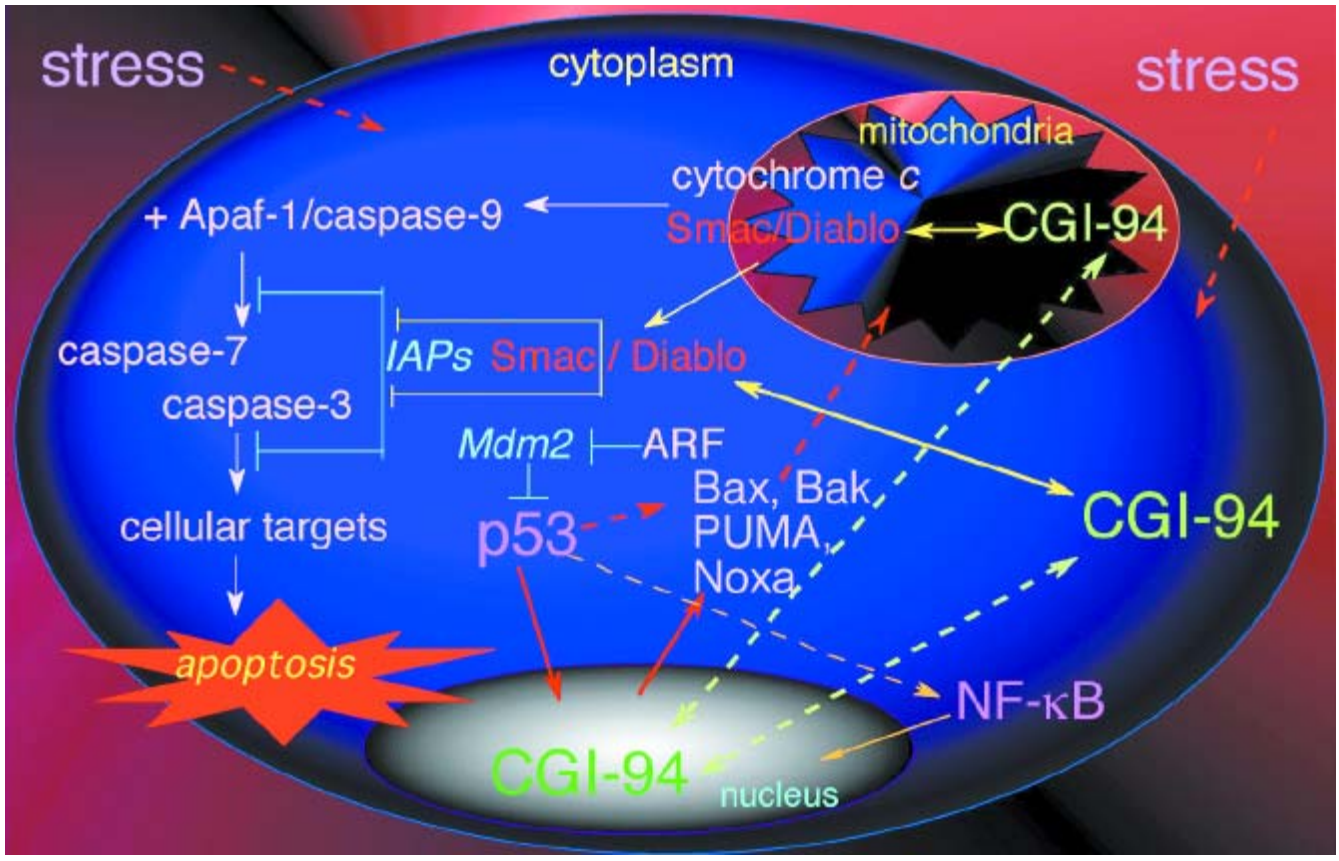

Figure 3. Schematic illustration of CGI-94-activity in apoptotic processes induced for instance by stress or growth factor deprivation.

Interestingly, it has been demonstrated that cultured Smac–(-/-) cells from Smac-deficient mice responded normally to all apoptotic stimuli applied. There were also no detectable differences in Fas-mediated apoptosis in vivo. Those data strongly suggest the existence of a redundant molecule or molecules capable of compensating for a loss of Smac function[14].

Here we show that the new protein CGI-94 – which is involved in early stage of AD[4] and in mediating neuronal cell death[5] – co-localizes with the 'master regulator of apoptosis' Smac/Diablo[11-13] and, taking into account its ability to enter the nucleus, cytoplasm and mitochondria[4], CGI-94 can mediate reciprocation between the nucleus and mitochondria as described for the crucial pro-apoptotic transcription factors p53 and TR3[15].

The ribosome is a ribonucleoprotein (RNP) complex dedicated to protein synthesis – it stands at the center of the translational machinery. The active U3 particle is a large nucleolar RNP and has been called the small subunit (SSU) processome because its components are required for pre-18S rRNA processing. There are at least 28 proteins in the SSU processome and all of the proteins essential for growth are required for 18S rRNA biogenesis. The SSU processome functions both in pre-rRNA cleavage and as a pre-rRNA chaperone, thus it functions in the earliest steps in ribosome biogenesis.

Systematic deletion (with CGI-94-specific siRNA) of CGI-94 (Utp11), as a part of the SSU processome, results in a non-viable phenotype in our neuronal cell culture system.

Interestingly, two proteins known to be involved in promoting apoptosis in mammalian cells have been identified as components of the mammalian mitochondrial ribosome: a) the death-associated protein 3 (DAP3)[16] and b) the programmed cell death protein 9 (PDCD9)[17].

The apoptosis-related protein DAP3 was found to be a component of the small subunit of mitochondrial ribosome (mitoribosome), indicating a new function for the mitoribosome in programmed cell death. DAP3 is a positive mediator of tumor necrosis factor-α-, Fas- and IFN-γ-induced cell death[18]. DAP3 is implicated as a positive mediator of these death-inducing stimuli. Over-expression of DAP3 in mammalian cells induces cell death. It functions downstream of the receptor signaling complex and (upstream to some caspases) its death promoting effects depend on caspase activity. DAP3 is a positive mediator of cell death and not a suppressor of a protective arm of signaling[18,19].

Moreover, it could be shown that ribosomal proteins were relocalized and expressed at the cell surface and may serve as autoantigens during apoptosis. There are recent reports demonstrating that ribosomal proteins are released from the ribosome and relocated in apoptotic cells. In one case, the ribosomal protein S19 is secreted from apoptotic

cells and serves as a chemoattractant specific for macrophages. Therefore, externalized ribosomal proteins could serve as markers for recognition of apoptotic cells by phagocytic cells. Thus, ribosomal proteins have extra-ribosomal functions[20].

Taking these recent findings into account, CGI-94 seems to be a potential ribosome-associated regulator of neuronal cell death. Particularly, our recent data have shown that CGI-94 is localized in the nucleolus, mitochondria, at the cell surface and even extra-cellular and interacts with the 'master regulator of apoptosis' Smac/Diablo[4,5]. A 'discerning eye' of the cell has to keep a balanced expression level of CGI-94 as a low levels leads to cell death due to inhibition of ribosome biogenesis and a high level may lead (as in the case of DAP3) to apoptosis due to activation of Smac/Diablo[5].

In conclusion, CGI-94 appears to be involved in the control of apoptotic processes during neuronal degeneration[4]. Thus, further knowledge about age-related changes in apoptosis might become therapeutically helpful in many areas of medicine including neurodegenerative diseases such as Alzheimer's disease.

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Quetiapine Effectively Reduces Psychotic Symptoms in Patients with Lewy Body Dementia: an Advantage of the Unique Pharmacological Profile?

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Abstract

Hallucinations, delusions and nighttime agitation are prominent psychiatric symptoms of Lewy body dementia (LBD). Their treatment is difficult because most patients often respond poorly to or develop severe side effects from antipsychotic drugs that block dopamine receptors. We studied effects of quetiapine, a novel antipsychotic, on psychotic symptoms in LBD patients. Quetiapine, at daily doses ranging between 25-150 mg, significantly reduced psychosis symptoms measured by the Neuropsychiatric Inventory and BPRS at six and twelve weeks of treatment. There was no significant change in extrapyramidal symptoms. These findings suggest that quetiapine may be an effective and well-tolerated therapeutic agent for the treatment of psychosis associated with LBD.

Keywords: Lewy body dementia, quetiapine

Lewy body disease (LBD) is a neurodegenerative disorder clinically characterized by dementia syndrome, fluctuations in cognition, psychosis, and mild parkinsonism[1,2]. Characteristic neuropathological findings of LBD resemble those of Parkinson's disease and show a widespread distribution of halo-enclosed cytoplasmic bodies (Lewy bodies) in the brainstem nuclei as well as in cortical and sub-cortical regions[3,4]. Based on the pathological findings, LBD is thought to account for as many as 27% of all dementia cases[5]. One of the greatest obstacles for treatment of psychosis associated with LBD is patient intolerance of antipsychotics and unwanted severe side effects. Neuroleptic sensitivity affects approximately 50% of LBD patients and represents a dangerous and potentially fatal facet of treatment[6,7]. Unlike other atypical agents, quetiapine has minimal affinity for dopamine and acetylcholine receptors, which make it an attractive candidate to treat psychosis in LBD patients. In studies involving patients with psychosis associated with Parkinson's disease, quetiapine has been shown to be effective in treating the psychosis without exacerbating extrapyramidal symptoms[8,9]. In this study we examined the possibility that quetiapine can be effective in treating the LBD-associated psychosis with a minimal risk of severe side effects.

Subjects

Ten consecutive male subjects between the ages of 58 and 78 were recruited from the outpatient psychiatry department of the Long Beach VA HCS Memory clinic. The diagnosis of LBD was made using CERAD criteria (the consortium to establish a registry for Alzheimer's disease), which have been shown to be a specific and sensitive measure available for the diagnosis of LBD[10] (Table 1). Inclusion criteria allowed for male and female patients between the ages of 55 and 85 who had a caregiver regularly present and were able to comply with the study protocol. Patients with a diagnosis of major depression, schizophrenia, bipolar disorder, psychoactive substance dependency or Parkinson's disease were excluded. In addition, patients with seizure disorder, metabolic disorders

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and those with LBD currently benefiting from an antipsychotic medication were also excluded. Patients who were taking an antipsychotic (other than quetiapine) without an appreciable clinical benefit or having significant side effects were included after a one-week washout period. Permitted concomitant psychotropic medications were benzodiazepines, antidepressants and cognitive enhancers.

Procedures

The 12-week dose titration study consisted of a screening interview and baseline assessment followed by visits every three weeks for twelve weeks (Table 2). The extensive battery of tests at the screening visit, medical history review and clinical interviews were used to eliminate conditions of exclusion. At baseline patients were started on a daily quetiapine dose of 25 mg except for one patient who was washed off risperidone and was started on 50 mg quetiapine a day, and another, that was started on 12.5 mg a day dose. The dose was increased if clinically required every three weeks until the patient was symptom free or no further reduction in symptoms could be achieved. The Neuropsychiatric Inventory (NPI)[11] was the primary outcome measure and was administered to the caregiver to assess 12 behavioral domains. Secondary outcome measures consisted of behavioral, (Brief Psychiatric Rating Scale [BPRS], Geriatric Depression Scale [GDS], Functional Assessment Questionnaire [FAQ]) cognitive, (Mini Mental Status Examination [MMSE], Cambridge Automated Neuropsychological Test Assessment Battery[12] [CANTAB]) and motor (Simpson Angus Scale [SAS], Abnormal Involuntary Movement Scale [AIMS], record of falls) components. The NPI, SAS, AIMS, vital signs, and number of falls were recorded at each visit while at the six and twelve week visits all the outcome measures were administered as well as ECG and the laboratory panel. All data are shown as mean $\pm$ standard error of the mean (s.e.m.). Repeated measures ANOVA with Dunnett's correction for multiple comparisons was used for all comparisons unless indicated otherwise.

Table 1. CERAD criteria [Consortium to Establish a Registry For Alzheimer's disease] (Luis *et. al.*, 1999)

a.	Clinical Dementia rating scale score:	must be $\geq$0.5
b.	Any two of the following:	1. Delusions or hallucinations
		2. EPS signs
		3. Unexplained falls and/or changes in consciousness.
c.	One or more of the following:	1. Fluctuating course
		2. Poor response to Levodopa
		3. Cognitive symptoms > EPS
		4. Cachexia, dysphagia, depression or dysthymia
d.	Exclusion of other causes of dementia.	

Table 2. Procedures during 12-week treatment period.

SCREEN	BASELINE Week 0	VISIT #1 Week 3	VISIT #2 Week 6	VISIT #3 Week 9	VISIT # 4 Week 12
History	NPI	NPI	NPI	NPI	NPI
P.E.	SAS	SAS	SAS	SAS	SAS
CT or MRI	AIMS	AIMS	AIMS	AIMS	AIMS
Chest x-ray	FAQ	Vitals	FAQ	Vitals	FAQ
ECG	CGI	Adv. events	CGI	Adv. Events	CGI
Vitals	MMSE	Rec.of falls	MMSE	Rec. of falls	MMSE
Labs	CANTAB		CANTAB		CANTAB
Urinalysis	BPRS		BPRS		BPRS
Rec. of falls	GDS		GDS		GDS
	Vitals		ECG		ECG
	Rec. of falls		Vitals		Vitals
			Labs		Labs
			Urinalysis		Urinalysis
			Adv. Events		Adv. Events
			Rec. of falls		Rec. of falls

Results

Ten male patients of an average age of 73±4 years enrolled and eight completed the study. One subject was withdrawn from the study after developing a bladder infection that, although deemed not related to the study medication, resulted in the patient being hospitalized and administered another antipsychotic in place of the study medication by an admitting physician who was unaware of the study. Another patient withdrew his consent after taking the first dose of study medication because of a reported exacerbation of symptoms. The average daily dose of quetiapine at baseline and 12 weeks was 26.3 and 51.7 mg respectively. At the conclusion of the 12-week treatment period 2 patients remained at the starting dose of 25 mg a day, 4 patients were receiving 50 mg, one patient was at 100 mg, and another patient was receiving 150 mg. No significant changes occurred in body weight, laboratory values, ECG or blood pressure over the treatment period. There was a mild increase in heart rate from a baseline average of 70 beats/min. to 77 beats/min. at 12 weeks (Table 3). Two patients reported somnolence and weakness; one patient experienced mild extrapyramidal symptoms (EPS) in the right arm and one patient reported chills.

Table 3. Average changes for health related measures.

	Baseline	6 weeks	12 weeks
Blood pressure	133/78	131/74	134/77
Heart rate	70	72	77
Weight	183	188	183
QTc	408	406	408

Table 4. Quetiapine effects on outcome measures. Repeated Measures ANOVA with Dunnett's correction for multiple comparisons. Calculations were based on 8 patients who completed the study.

Measure	P value
NPI	0.025
BPRS	0.001
SAS	0.182
AIMS	0.655
MMSE	0.285
FAQ	0.967
GDS	0.430
Sleep	0.059
QTC	0.964
Caregiver Distress	0.106

Analysis of overall NPI scores revealed a statistically significant (p=0.025) reduction from the baseline at 3, 6, 9, and 12 weeks (Table 4). Reduction was also found in NPI hallucinations, delusions, irritation, and agitation sub-scale scores at both six and twelve weeks (Figure 1). Caregiver distress scores on the NPI dropped by an average of 45±16% at six weeks and 43±21% at twelve weeks but the changes were not statistically significant (Table 4). In addition, there were marked reductions in sleep disturbances, apathy and aberrant motor behavior (Figure 1). These changes in NPI scores were paralleled by statistically significant changes in BPRS scores (normalized to the baseline) at six (27±12%) and twelve (29±13%) weeks (Table 4). Furthermore, combined hallucinations, suspiciousness, irritability and agitation sub-scale scores of the BPRS were reduced by 36±11% (p=0.03, t-test) at six weeks and 46±13% (p<0.02, t-test) at twelve weeks. The major reductions were seen in hallucinations and suspiciousness scores (Figure 2). Extrapyramidal symptom scores as measured by the AIMS (14±84%) and SAS (18±12%) did not change significantly (p>0.05) or clinically during twelve weeks of quetiapine treatment. CGI scores at six weeks showed 2 patients with no change, 5 patients with minimal improvement and 1 patient with much improvement. At 12 weeks 1 patient was minimally worse, 4 patients were minimally improved and 3 patients were much improved.

There were no significant treatment effects on cognitive or functional measures nor was there any change in depression as measured by the GDS (6±21%) scores. A mild but non-statistically significant (p>0.05) improvement was found on MMSE (8±29%) and FAQ (5±18%) scores. Only three subjects could complete the CANTAB assessment, which showed a mild improvement in the number of problems that patients were able to solve in a set amount of time. There was a strong trend in sleep improvement (Table 4) and those 5 patients who had a measurable sleep disturbance at the baseline, showed a statistically significant treatment effect (p<0.05, Figure 1).

Discussion

In the present study, quetiapine, at daily doses between 25 and 100 mg dramatically reduced psychiatric disturbances in patients suffering from Lewy body dementia without increasing extrapyramidal symptoms. NPI scores for delusions, hallucinations, agitation, and irritation were markedly reduced at six and 12 weeks. Other noticeably reduced NPI scores were anxiety, agitation, apathy, aberrant motor behavior, and caregiver distress. There was a significant reduction in sleep disturbances in those patients who had such disturbances at the baseline. Despite these pronounced changes in measurement values, two patients did not show consistent improvement. Two subjects had overall increases in NPI scores (subjects A and B) caused by

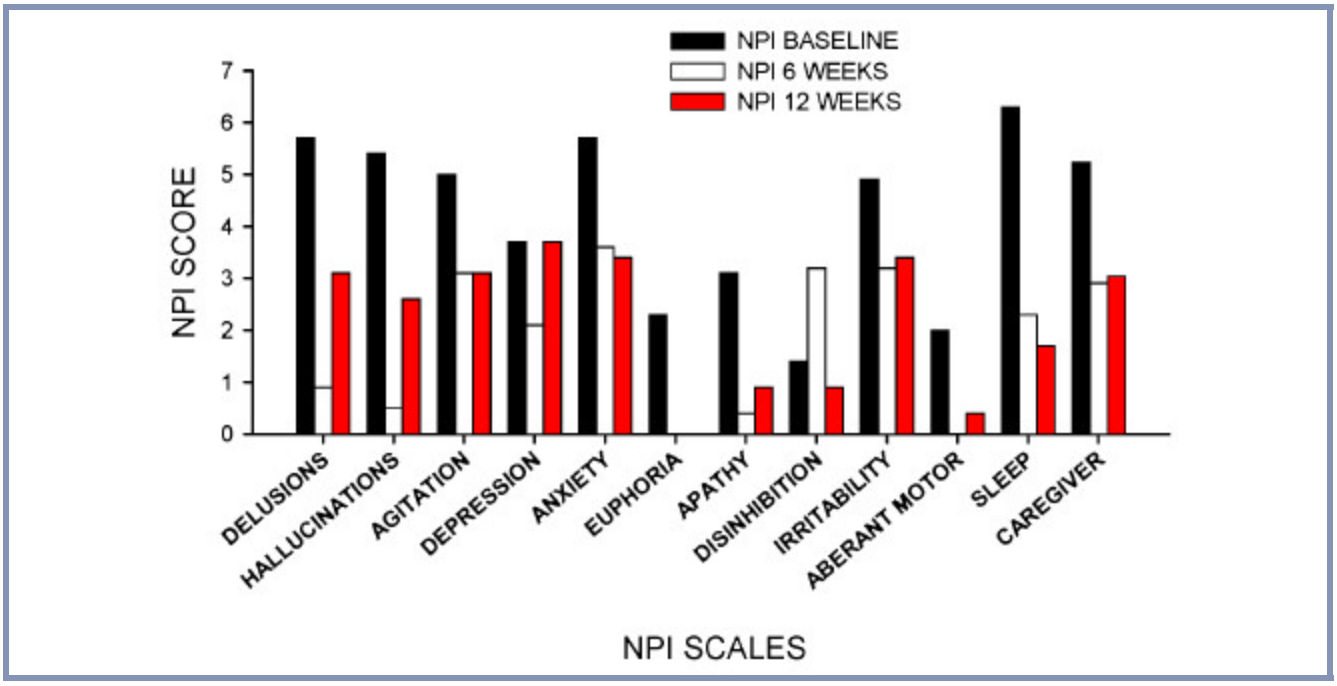

Figure 1. The quetiapine effect on Neuropsychiatric Inventory (NPI) individual scale scores.

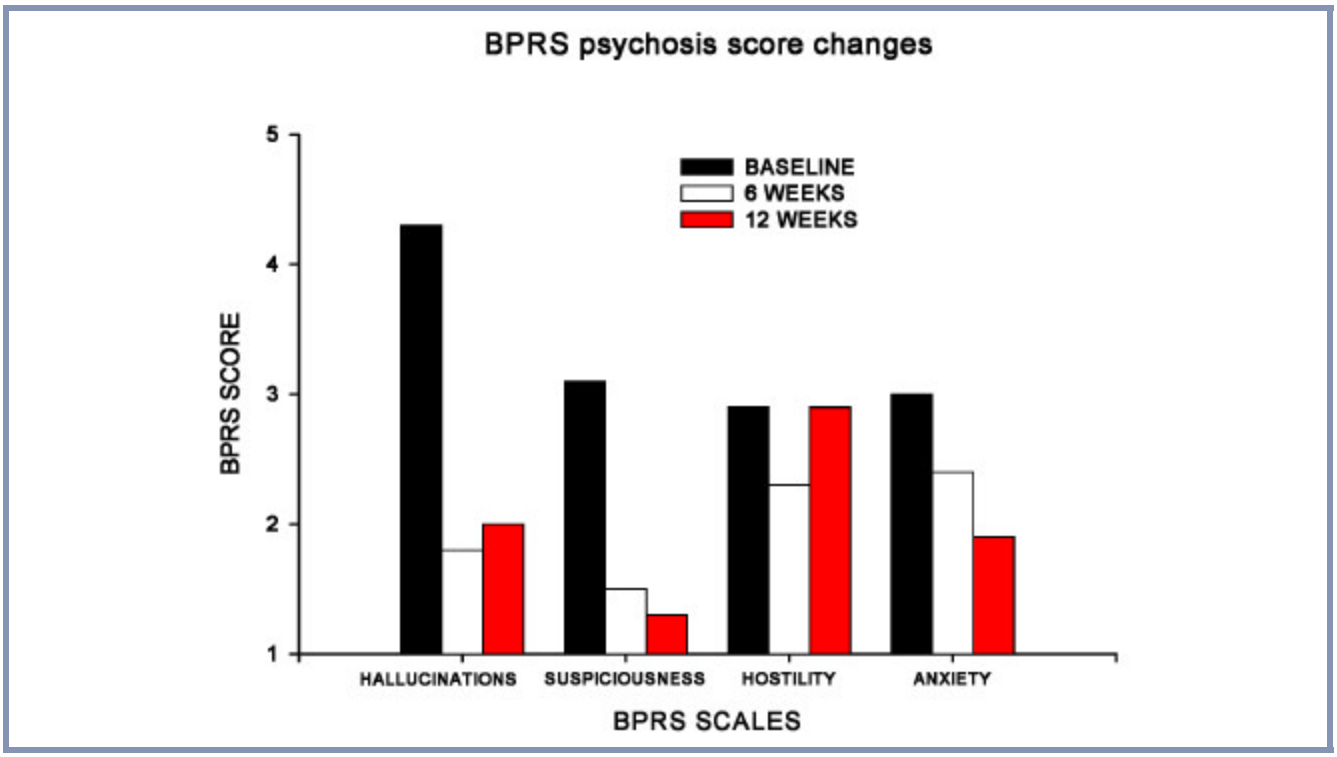

Figure 2. The quetiapine effect on Brief Psychiatric Rating Scale (BPRS) psychosis sub-scales (hallucinations, suspiciousness, hostility and anxiety).

increases in agitation and irritability and subject A had an increase in apathy and disinhibition measures; subject B had reductions in these domains. In addition, subject A had no change in hallucinations, delusions, depression, and anxiety and subject B had increases in all of these domains. This inconsistency in LBD patients is equally as difficult to predict as to explain but it may be reflective of the fluctuations in mental status typical of LBD patients as well as contributing to a somewhat low accuracy of available diagnostic criteria for LBD[13]. In the present study a mild

average increase observed on MMSE and FAQ scales did not reach statistical significance. Earlier studies involving LBD patients found that reductions in psychiatric disturbances were mirrored by improvements in cognitive functioning[14,15]. In these studies the improvements in cognitive functioning were though to be the result of reductions in psychiatric disturbances. NPI caregiver distress scores were also reduced by nearly half during the 12-week treatment period. At baseline, family members involved with the patients reported progressively escalating levels of emotional distress in relation to providing care for these patients. Stress reduction felt by caregivers early in the study may help to explain the initial reduction in NPI scores at 6 weeks and the eventual rebound at 12 weeks, likely reflecting caregiver difficulties in dealing with a demented patient. Nevertheless, NPI scores continued to remain well below baseline values throughout the study.

The reduction in motor dysfunction by 18% at the endpoint as measured by the SAS in the current study is consistent with quetiapine use in elderly patients with Alzheimer's, Parkinson's disease, and other disorders[16]. In these studies quetiapine decreased SAS scores while AIMS scores continued to remain at or near baseline values.

The open label aspect of the current study gives rise to questions of placebo effects, in relation to caregivers and patients. Although the type and magnitude of the symptom reduction in severe hallucinations, delusions and sleep dysfunction make the placebo effect less likely to be responsible. This possibility cannot entirely be excluded without performing larger placebo-controlled studies.

It is well known that LBD patients do not tolerate conventional antipsychotics with high affinity for dopamine receptors[7]. Several atypical agents have been tried but were only minimally effective in treating LBD symptoms and often had intolerable side effects. Olanzapine, when used with PD and LBD patients, was poorly tolerated and had little or no efficacy in treating the psychiatric symptoms of these patients[17]. Risperidone has also resulted in similar drawbacks when used with PD and LBD patients and the use of these agents has been recommended only as a short-term solution or not at all in PD patients with psychosis. Clozapine has been used with some success in LBD patients and while the risk of EPS with this agent is lower than the other atypical agents discussed, the risk of agranulocytosis and repeated blood draws make it an undesirable candidate for the treatment of LBD[18, 19]. Geodon, to our knowledge, has not been examined in this population.

The absence of EPS in LBD patients treated with quetiapine in the present study presents an interesting question about the pharmacological profile of this particular agent. Quetiapine has greater affinity for serotoninergic receptors than dopaminergic receptors, which may explain its distinctive benefit in this population whose dopaminergic system is believed to be affected by the disease process. At low doses, quetiapine is reported to have little or no D2 occupancy, which, aside from clozapine, makes it the only other antipsychotic with this profile[19]. Because of the increased risks associated with clozapine and the high rates of D2 occupancy of other antipsychotic medications, quetiapine appears to have a unique advantage in the pharmacological treatment of patients suffering from LBD.

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Major Fluctuations as the Principal Cause of Impairment in Daily Living Activities in a Case of Probable Lewy Body Dementia

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Abstract

Probable dementia with Lewy bodies (DLB) was diagnosed in a patient after a procedure that included neurological, neuropsychological, neuroimaging and sleep examinations. Fluctuations, a core diagnostic feature of DLB, appeared gradually in the patient, who presented periods of confusion alternating with near-normal functioning. The patient entered a cognitive rehabilitation programme that allowed to demonstrate how fluctuations impeded the performance of daily living activities, and how they prevented the benefit of the rehabilitation procedure. In summary, when our demented patient was well, she could learn and automate very quickly new activities of daily living. Conversely, when she was confused, she lost the benefit of previous learning, even for simple automated activities.

Keywords: fluctuations, cognitive rehabilitation, activities of daily living, neuropsychology, Lewy bodies dementia, Alzheimer's disease.

Introduction

Researches over the past years have shown the importance and the benefits of cognitive rehabilitation in the management of Alzheimer's disease[1-3]. However, such an approach has not been attempted in an other degenerative dementia.

Dementia with Lewy bodies (DLB) is claimed to be the second most common form of degenerative dementia in old age, after Alzheimer's disease[4]. Salient neuropsychological features are prominent attentional deficits and visuospatial dysfunction. The clinical presentation is characterised by fluctuating cognitive impairment, accompanied by complex visual hallucinations and mild extrapyramidal features[4,5]. Dementia plus one of those last clinical features is required to make a possible diagnosis of DLB, and two features are needed for a probable diagnosis of DLB. Other clinical signs supportive of the diagnosis include repeated falls, syncopes, transient loss of consciousness, neuroleptic hypersensitivity, systematised delusions, and non-visual hallucinations[6].

We present in this paper the diagnostic assessment and a trial of cognitive rehabilitation conducted in a patient with probable DLB. Important fluctuations with progressive increase of confusion constituted a major obstacle to rehabilitation of instrumental activities of daily living (IADL).

Case report

Patient's history

The patient GL was a 76-year-old, right-handed, woman who complained of progressive memory deficits for recent information and difficulties in word findings and in knitting. She had nine children who described also mild difficulties in spatial orientation, for washing and dressing, for using phone and household electricals, and for financial management. GL had 8 years of education and she had hold a farm with her husband. She was widowed and lived alone at home. Medical history comprised a replacement of aortic valve, atrial fibrillation and angor pectoris, retinal transient

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ischemic attack and surgery for right and left hip prosthesis. This last operation, when GL was 74 years old, caused a transient delirium with hallucinations, which were increased by classical neuroleptics. Progressive memory difficulties were reported and a first neuropsychological evaluation was performed. Visuo-spatial difficulties were observed in copying the Rey complex figure, and inhibition deficit was noticed in the interference condition of the Stroop Task[7]. On the other hand, despite memory complaints, episodic memory performance evaluated by the California Verbal Learning Test[8], and short-term memory evaluated by the digit span task were normal. GL spoke fluently, with no major word-finding difficulty. Her performance was normal in phonemic and semantic fluency tasks.

The next neurological examination was performed two years later when memory capacities got worse. The patient could remember information concerning children and grandchildren, but forgetting was obvious for activities of daily living. Consequently, medication was prepared each day by one of her sons, and help was required for cooking and cleaning the house. Use of the phone was problematic and help was necessary for administrative matters. For leisure's time, GL stopped knitting but she was still frequently going out for a walk. The patient was not completely aware of her daily living impairments. However, she reported that she was sometimes surprised to talk to herself in a mirror, and that she might mistake the TV remote control for her phone. An early stage of dementia was diagnosed, and complementary neurological, neuropsychological and laboratory investigations were undertaken. There was a suspicion of family history of dementia in her father.

Neuropsychological evaluation

GL obtained a Mini Mental State Examination (MMSE)[9] score of 16/30 and a score of 119/144 on the Mattis Dementia Rating Scale (DRS)[10]. The patient was also administered a standard battery of neuropsychological tests (see table 1). Her short-term memory, investigated by the forward digit span test and the Corsi block-tapping procedure[11] was normal. However, her visual short-term memory was impaired when investigated by the visual span of the Wechsler Memory Scale (WMS-R)[12] and by the span and supraspan task[13]. She showed normal performance in an episodic memory task, the Free and Cue Selective Reminding Test[14]. GL's performance in free recall was normal to weak compared to performance of normal elderly subjects. Her performance was normal when category cues were provided. On the contrary, a severe episodic memory deficit was observed at the CVLT[8]. GL's performance in the encoding phase and in free recall was low. Her score was better when category cues were provided, but remained abnormally low. The difference of results between this task and the Free and Cue Selective Reminding Test could be due to the nature of encoding that is free in the CVLT and forced in the Free and Cue Selective Reminding Test. GL spoke fluently, with no major word-finding difficulty. However, she showed deficit in a phonemic and semantic fluency task similar to the one performed two years earlier. Verbal comprehension, evaluated by the Token Test[15] was below the average. Difficulties were observed in several executive tasks. In the Stroop task[7], GL produced many errors in the interference condition, suggesting an inhibition deficit. In the Trail Making Test[16], the part B was stopped due to many errors, suggesting a flexibility deficit. An important deficit affecting the central executive of working memory was also observed in a Dual Task[17]. Visuo-spatial difficulties were revealed in copying the Rey complex figure, in the subtest "copy of geometric figures" from the *Contorsium to Establish a Registry for Alzheimer's Disease* (CERAD)[18] and in the Shapes subtest from the Doors and People Test[19], where GL could not reproduce the pictures. In summary, the neuropsychological assessment revealed mainly deficits in episodic memory for free encoding condition, in executive functions, and in visuo-spatial capacities.

Initial diagnosis

Taking into account GL's clinical history and the neuropsychological examination, a diagnosis of AD was first proposed. EEG showed an alpha rhythm intermixed with mildly abundant diffuse theta activities. Biological screening was normal. Magnetic resonance imaging (MRI) revealed mild leucoaraiosis in the left periventricular white matter. In spite of her cardiovascular history, there was no neurological argument for a diagnosis of vascular dementia. Hashinski ischemic score[20] was 2, related to nocturnal confusion and associated atherosclerosis. Thus, GL met criteria for probable AD[21]. Furthermore, a positron emission tomography (18FDG-PET) scan showed a decrease of metabolism predominant in middle prefrontal and parietal areas, consistent with a diagnosis of Alzheimer's disease. A trial of acethylcholinesterase inhibitor therapy was started. With this diagnosis of probable AD, the possibility of a cognitive rehabilitation at the day care centre of the University Hospital in Liege was discussed with GL and her daughters, and they gave consent to take part in the day care programme.

Initial evaluation in the cognitive rehabilitation centre

On admission, the complaints expressed by the patient and her daughters concerned memory deficits, bad use of household electricals and telephone, reduction of activities and presence of some sadness. Few hallucinations were initially reported, but they were not frequent and they could not be precisely described. At the Neuropsychiatric Inventory (NPI)[22], disorders noted by GL's daughters were,

Table 1. Assessment of GL's general neuropsychological abilities when 76 years old

	Raw score	Normative data mean (SD)	Performance descriptors
Working memory			
Digit Span	5	4.98 (0.97)	normal
Block Tapping Test	4	4.24 (1.11)	normal
Dual Task – Performance Decrease	60.63	92.43 (16.40)	impaired
Episodic Memory			
Free and Cued Selective Reminding Test			
Free Recall 1	3	8.7 (2.1)	impaired
Free Recall 1 + Cued Recall 1	12	14.8 (1.4)	impaired
Free Recall 2	8	10 (2.9)	normal
Free Recall 2 + Cued Recall 2	15	15.2 (1)	normal
Free Recall 3	8	9.9 (2.3)	normal
Free Recall 3 + Cued Recall 3	15	15.5 (1)	normal
Delayed Free Recall	8	10.4 (2.9)	normal
Delayed Free + Cued Recall	15	15.03 (0.9)	normal
Recognition	16	15.8 (0.5)	normal
CVLT			
Sum recalls 1 to 5	20	47.31 (9.54)	impaired
Recall list B	0	5.83 (2.47)	impaired
Short free recall	1	8.69 (2.95)	impaired
Short cued recall	5	10.24 (2.36)	impaired
Delayed free recall	3	9 (3.09)	impaired
Delayed cued recall	5	10.45 (2.57)	impaired
Intrusions	2	7.1 (5.92)	normal
Doubles	0	5 (4.27)	normal
Recognition	16	14.66 (2)	normal
Rey Complex Figure – Delayed Recall	2.5/36	< Percentile 10	impaired
Instrumental functioning			
Forms copy (CERAD)	6	8.8 (1.9)	weak
Rey Complex Figure - Copy	6/36	< Percentile 10	impaired
Clock Drawing	5	> the cut-off score of 2	impaired
Oral language			
Naming Battery	53/64	57.9 (4.97)	normal
Phonemic Fluency Task (P)	6	14.71 (7.56)	weak
Phonemic Fluency Task (R)	7	13 (6.97)	weak
Phonemic Fluency Task (V)	5	11.42 (4.98)	weak
Semantic Fluency Task (animals)	11	25.78 (5.32)	impaired
Attentional functioning			
Code (WAIS-R)	4	10 (3)	weak
Trail Making Test			
Part A	140	113.9 (37.73)	normal
Part B	Stop	242 (69.16)	impaired
Stroop task			
Denomination Time	140	81.83 (18.27)	impaired
Errors	4	1.33 (1.13)	impaired
Reading Time	90	56.46 (13.46)	impaired
Errors	6	0.17 (0.38)	impaired
Interference Time	440	187.75 (72.32)	impaired
Errors	28	6.08 (5.53)	impaired

in decreasing order: night-time disturbances, eating changes, agitation/aggressivity, delusions, sadness, hallucinations (difficult to distinguish from somnambulism) and to a lesser extent, apathy and euphoria. The short-version of the Geriatric Depression Scale[23] administered to the patient revealed the presence of a slight depression (a score of 6/15, just above the cut-off score of 5/15). Some deficits were observed in a scale assessing basic and instrumental everyday life activities for hygiene, nutrition, financial management, reading, writing, phone use, and conversation initiation[24]. An exploration of leisure and other activities that GL had preferred in the course of her life revealed three activities practised until recently: knitting, cooking, and frequenting old people's club. Those activities were stopped or reduced during last years, because of incapacity, loss of interest and interdiction of driving.

Rehabilitation contract

A 3-month rehabilitation programme was proposed to GL and her daughters, with a frequency of two half-days a week. The objectives were: (1) to reduce the consequences of memory lapses by promoting the use of a simplified calendar; (2) to reduce GL's anxiety in cooking by introducing adapted help; and (3) to automate the phone use.

The simplified calendar was a large board with one case per day. The children coming for helping GL had to note the different appointments and activities to perform whereas GL had only to consult it. This consultation was trained at the day care centre by role playing using the space retrieval technique[25,26]. In addition, GL's daughters received instructions to encourage the use of this calendar at home.

For the phone, the initial evaluation showed transcribing and memory difficulties: GL didn't know by heart numbers of her children, and could not correctly reproduce numbers on the phone. An adapted phone was proposed. This phone had twelve large keys containing pictures of people to call. Number was automatically composed when a key was pressed. The utilisation was trained at the day care centre by space retrieval technique and by errorless training[27].

For cooking, the initial objective was to automate the use of a timer to reduce GL's anxiety, because she never knew if she had switched off her cooker. So, we wanted to create an automation: when GL heard her timer, she had to switch off her cooker.

Occurrence of fluctuations

We gradually observed longer periods of confusion. Those confusing episodes were first short and not very obvious to describe since the patient lived alone, but they became more pronounced over the weeks and could be observed in the cognitive rehabilitation centre. At that time, GL's speech was mostly incomprehensible: she didn't always finish sentences and she spoke incoherently. She was also very sensitive to interference and could be impulsive. Even simple things were impossible to do. At home, those periods of confusion were frequently observed by the children. In the same way, complex visual hallucinations were reported to be more frequent and tiring at home. For example, GL could see and precisely describe her son having a car accident. She could see her grandchildren sitting on the sofa and she could decide to phone her daughter because the grandchildren refused to talk. During one of those episodes of confusion, GL was unable to turn off her electric cooker and had to call her granddaughter. Consequently, we decided to reduce our objectives for cooking. Effectively, it was more important for GL's safety to be able to turn off her cooker than to use a timer, especially when she was confused.

Adapted rehabilitation programme for cooking

We decided to automate the gesture of turning off the cooker. For this, we realised a jerry-built cooker similar to GL's one and designed an automation procedure without errors[27] using the space retrieval technique[25,26]. *Errorless learning* is more a principle than a real technique. It consists to repeatedly expose the patient to the correct response rather than asking him to guess or to explicitly retrieve it. Effectively, an amnesiac is not able to remember his mistakes and learns them as he learns the correct responses. The *space retrieval technique* prompts recall of information over increasingly longer retention intervals. So, specific information to remember is given to the patient and immediate recall is asked. If the patient gives the correct response, the intertrial interval is increased systematically (for example 5, 15, 30, 45 seconds…). Following a recall failure, the interval is decreased to that of the previous trial. Studies showed that over a critical interval, if the patient was able to successfully recall information, this information seemed to be in long term memory[3]. The intertrial intervals used were 0, 15, 30, 60, 90, 120, 180 and 240 seconds. We considered that the information was learned when the patient could remember the correct information two times in succession at four minutes delay, during two consecutive sessions. We hoped that the automatism so created would be preserved even during periods of confusion.

Before beginning the work of automation, we performed baseline evaluations to characterise the difficulties. During three consecutive sessions, we asked GL to realise different exercises evaluating her knowledge about correspondence tackles-buttons, buttons-tackles, and about how to switch off the different tackles. This evaluation showed difficulties everywhere. So, we decided to automate the gesture of turning off every tackle, independently of which were switch on or not.

In front of her fictive cooker, we asked GL to turn off the cooker. We began with four pans that were put on the tackles turned on. The instruction was "Everything is

cooked" and GL had to turn off the buttons. If GL seemed to do a mistake, we stopped her with verbal or physical helps and the interval was decreased to that of the previous one. Major fluctuations were observed between sessions. Those fluctuations are illustrated on figure 1.

As observed on the different figures, when GL was well, her performance was rapidly perfect and showed that she could correctly use her electrical cooker. Conversely, when she was confused, she could not turn off the tackles and the space retrieval trials reached a plateau with low performance. Her mistakes were to forget one button, to

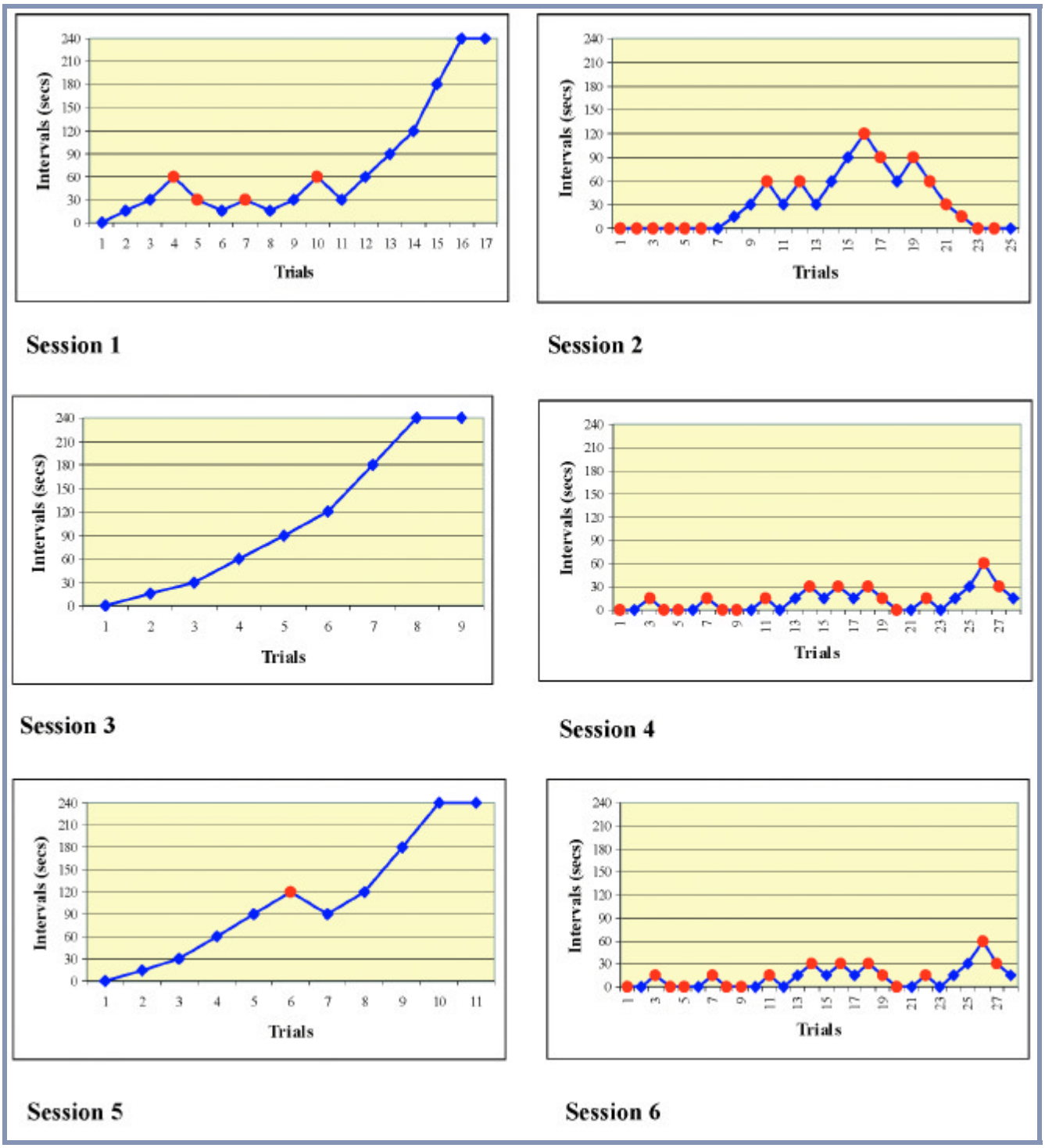

Figure 1. Data per session concerning the cooking activity with the space retrieval technique. ● = recall failure, ◆ = successfully recall.

bring the button on another number than 0, or to switch on a tackle previously switched off. So, the automation created when GL was well was not preserved when she was confused. Moreover, it is possible that the difficulties noticed during baseline evaluation were due to minor fluctuations appearing and disappearing rapidly.

The same fluctuations were observed for the calendar and for the phone's use. When GL was fit, she performed well, and when she was confused, no automation was possible. These results and observations showed that the techniques usually used for cognitive rehabilitation seemed to be inapplicable and that adaptations should be necessary in a subject experiencing such major fluctuations.

Further evaluation of attention

Variations in attention and alertness are common in DLB and they might explain fluctuations[5,28,29]. Walker *et al.*[28] have showed that DLB patients experienced greater variability than AD patients in computerised attentional task. Our patient GL performed twice the "Alert Phasic" subtest from the Test Attention Performance battery (TAP)[30]. One was realised when she was clinically "well", and one when she seemed confused. This task follows a ABBA design. In part A, subject has to press a key as quickly as he can when a cross appears on the screen. It measures simple visual reaction times. In part B, a sound is preceding the cross and the subject has to press the key as quickly as he can when the cross appears on the screen. The difference between the two conditions assesses alertness, that is the ability to enhance response readiness following a warning stimulus. Different measures are available: average reaction time, average standard deviation for reaction times, number of omissions. On both examinations, GL's standard deviations were always below the percentile 1, showing an significant variability in reaction times as described by Walker *et al.*[28]. This variability is observed in figure 2. Only number of omissions differed between the two sessions: when she was confused, GL omitted 24 hits, against 4 when she was well (see on figure 2 the numbers of items necessary to accomplish the task). This could reflect laps in arousal during confusing state.

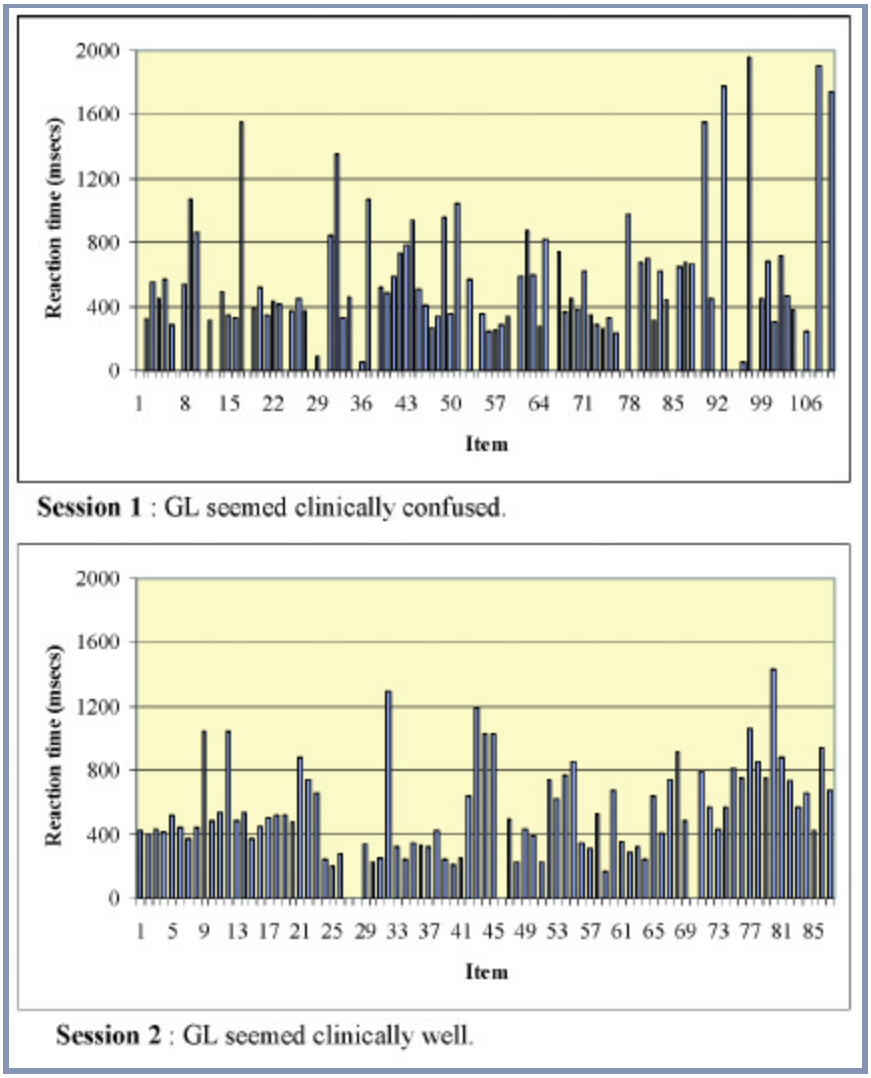

Figure 2. Data for the test "alertness" from the TAP battery. This test is composed of four conditions (two without a auditory warning signal and two with) that have been brought together on the figures. In abscissa are the numbers of items required for accomplishing the task, each condition needed the realisation of 20 items. Omissions were replaced by other items. In ordinate are the reaction time for each item realised.

Revised diagnosis

Progressively, a mild bradykinesia, major recurrent fluctuations (confirmed during cognitive rehabilitation), and tiring well-formed visual hallucinations did appear in our patient, and a diagnosis of DLB became probable[5].

Confusing episodes became so important and disabling that GL had to be hospitalised, four months after her admission in the cognitive rehabilitation centre. Moments of remission became rare. She could not stay alone: she didn't sleep anymore, she didn't eat correctly, she had tiring visual hallucinations and she was a menace for herself. During her stay at the Hospital, complementary examinations were performed, notably concerning her sleep. She indeed was reported to have night-time disturbances as acute nightmares, "agitation", and sometimes visual or auditory hallucinations. Day time EEG recording showed diffuse slowing of activities. An overnight video polysomnogram revealed excess abnormal tone during REM sleep, as already described in DLB[31].

Discussion

We present here the evaluation and an attempt of four month of cognitive rehabilitation in a patient with progressive but rapid evolution of probable DLB.

Diagnosis

The diagnosis of DLB is probable but not definite. However, GL presented the three clinical features which are considered to be the hallmarks of DLB: complex visual hallucinations, major fluctuations, and mild parkinsonism[5,32,33]. Moreover, she once presented a hypersensitivity to classical neuroleptics and a sleep study demonstrated REM sleep behavior disorder (RBD) that constitute additional diagnostic features[6,31].

From a neuropsychological perspective, attention, executive functioning, problem solving and visuo-spatial skills tend to be impaired more severely in DLB than in AD, whereas recent memory may be fairly well preserved in the early stages of DLB[4,5,34]. Working memory and cognitive reaction time seem also to be disproportionately impaired in patients with DLB[35,36]. The neuropsychological deficits observed in GL were consistent with a diagnosis of DLB (see table 1): early preserved episodic memory, impairment of executive and attentional functioning, disturbed visuo-spatial skills, and slowdown of information processing[35,36].

EEG and sleep studies

Some studies have evaluated the role of EEG in the diagnosis of DLB. Briel *et al*.[37] showed that the key findings were a loss of alpha activity, presence of temporal lobe slow wave transients and a strong correlation between the presence of these temporal slow waves transients and a clinical history of loss of consciousness. In addition to generalised slowing, sharp or triphasic waves may be a characteristic features. Idiopathic REM sleep behavior disorder (RBD), as observed in our patient, was also described in DLB: characteristic observations are loss of normal atonia demonstrated by increased tonic electromyographic activity and sometimes violent behavior that may represent acting out of dreams[31].

Hallucinations

Hallucinations were essentially visual, complex, well-formed and they predominantly concerned known peoples, still living or not, as described in the literature on DLB[5,38]. Classically, hallucinations may particularly occur during periods of diminished consciousness, but here, they appeared during night or day, lasted several hours, and didn't disappear even when GL realised that they might not be real. In the literature, hallucinations may be temporarily relieved by increased environmental stimulation such as it occurs during a clinical interview or after a move into group living[5], but here nothing could predict their appearance or make them disappear.

Bradykinesia

Mild parkinsonism consisted of bradykinesia, which is frequently described in DLB[32]. This feature of parkinsonism was not present at the beginning of the disease and appeared as the pathology progressed. Other common findings in the literature are hypophonic speech, masked facies, stooped posture and a slow and shuffling gait[5]. Those features are milder in DLB than in Parkinson's disease and the classical triad of tremor, akinesia, and rigidity is not likely to appear[39]. Heyman et al.[32] showed that the coexistence of multiple (two or more) extrapyramidal features was more common in DLB than in AD but that only bradykinesia was significantly more frequent in DLB.

Fluctuations

Fluctuations became more pronounced and frequent with the progression of the disease. Episodes of confusion alternated with periods of remission, which became sparse and finally non-existent. This was the reason why GL was hospitalised and then conducted in an old people's house. Such fluctuating course and rapid progression is described in patients with DLB[40]. The DLB consensus diagnostic criteria[5] state that fluctuating cognition (FC) is based upon "pronounced variations in attention and alertness" and is associated with episodes of disturbed consciousness. Excessive daytime drowsiness with transient confusion on waking is not uncommon and may be accentuated by an non

stimulating environment. In contrast, DLB patients may show improved performance in response to environment novelty and increased arousal[5]. For others, fluctuations are coded if marked variations in memory, emotion or behaviour are reported[6]. In this way, Byrne *et al*.[41] reported a patient experiencing so major fluctuations that she varied from being mute to being capable of carrying on a conversation. Those fluctuations can be described as periodic shifts in the level of arousal, ranging from episodes of lucidity to reduced awareness and even stupor (Ballard *et al.*, 2001). For the later authors, there is a significant association between fluctuating cognition and fluctuating attention. That's why Papka *et al.*[39] spoke of episodes of going blank or "switching off": the fluctuations are not restricted to cognitive functioning but they are generalised to other mental, physical and behavioral capacities. A variety of techniques have been proposed to assess fluctuating cognition or attention: 1) the use of questionnaires and diary filled by a reliable informant and 2) the use of psychometric procedures including computer-based tasks that are sensitive to the attentional dysfunction characteristic of DLB[4,28,35,42]. Variations in attention and alertness were also observed in GL during computerised attentional task from the TEA[30]. The fluctuating episodes were principally observed between sessions, but they were perhaps present within a single session, since the periodicity and amplitude of fluctuations are variable between subjects and for the same subject[5]. They can occur rapidly (lasting minutes or hours) or slowly (weekly or monthly).

Fluctuations during the cognitive rehabilitation programme

The objectives of the rehabilitation contract were to reduce the occurrence of memory lapses by promoting the use of a simplified calendar, to reduce GL's anxiety in cooking by introducing adapted help and to automate the phone use since GL was living alone and needed to be able to use her phone to contact her children. Those objectives were tackled with the techniques generally used for cognitive rehabilitation in dementia such as errorless learning[27] and space retrieval technique[25,26]. During the programme, objectives were reduced because new difficulties appeared in GL. Nevertheless, automation in the use of the different equipments was not possible. As showed in figure 1 for cooking, the fluctuations experienced by GL prevented the automation of simple activities. Effectively, when she was well, GL automated very well and very quickly the gestures we wanted to teach her (turning off all the tackles of her electric cooker, see trial 1). Conversely, when she was confused, no automation was possible and GL was not able to switch off her cooker. The same pattern was observed for the phone use and for the calendar. So, the fluctuations constituted the main cause of failure of classical cognitive rehabilitation for essential daily living activities. Moreover, the fluctuations affected GL's motivation for rehabilitation: when she was well, she thought she didn't need rehabilitation because she saw she could do many activities. Conversely, when she was confused, everything was too difficult for her and the objectives had to be simplified. She was not always conscious of her deficits and difficulties due to her confusing state.

Conclusion

All these elements showed that our cognitive rehabilitation programme was not adapted for demented patient experiencing major fluctuating confusion and a rapid progression of the disease. Indeed, cognitive rehabilitation in dementia is based on preserved abilities and optimisation factors allowing to enhance the performance of the patient, and GL presented periods of "switching off" where nothing in cognitive functioning seemed to be preserved. Cognitive difficulties during episodes of confusion seemed to be important and generalised to all domains required to perform daily living activities.

A beginning of solution could be to share cognitive rehabilitation. During confusing moments, no work seems possible with the patient. So, this time could be allocated to family education: how to face up to disease, how to manage stress, how to optimise communication with the patient. Explanations on hallucinations, fluctuations and the disease in general could be provided. During remission moments, intensive cognitive rehabilitation could be applied with the patient. The caregiver would be determinant in the choice of the rehabilitation goals: they should be based on problems present during remissions moments, when the patient is not confuse. This requires a good comprehension of the disease, and an constant observation of the patient by the relatives. All of this could help to manage symptomatology and cognitive deficits observed in dementia with Lewy bodies. But more research are needed to understand those remission moments and the learning realised during them: is it maintained from one to other periods, or is new learning lost after confusing moments? Research is also needed to understand confusional state in DLB and to determine if preserved capacities do exist during those moments of "switching off", with the additional difficulty to determine and foresee when and why exactly the patient becomes confuse.

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Diagnostic Work-up of Dementia – a Survey among Norwegian General Practitioners

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Abstract

Background. **To assess the management of patients with dementia in general practice, and to get the general practitioners (GPs) views on necessary changes for improving the diagnostic work-up of dementia in primary health care.**

Method. **A postal questionnaire was sent to all GPs in Norway (N=3604).**

Results. **After two reminders, the response rate was 55%. The assessment carried out by the GPs included in most cases a clinical examination and an interview with the patient. Only 23% made always use of formal cognitive tests. The most common trigger for detecting signs of dementia in their patients was when the carer alerted the doctor (50%). More than 85% of the GPs agreed that GP should play a central role in diagnosing a patient suspected of dementia. However, only half of them found the conditions for this work-up sufficient today. Improved collaboration between various health professionals working in primary health care, better theoretical and practical knowledge, better financial reimbursement and the wider availability of specialist services were suggested to improve the assessment of dementia in primary health-care.**

Conclusion. **According to this study Norwegian GPs wish to play a major role in the assessment of patients with suspected dementia. However, the assessment of these patients today by GPs is not sufficient according to current criteria. The GPs have suggestions for improving the diagnostic work-up in general practice.**

Keywords: family physicians, dementia, questionnaires, caregivers

Introduction

Dementia is infrequent before the age of 65, but after that its prevalence increases rapidly. The prevalence is estimated to be 15% in persons aged 75 and above, and more than 30% in persons aged 90 years and above. In the Gothenburg and the Rotterdam studies, the annual incidence rate was estimated to be 14.8 new cases per 1000 in persons 65 years and older[1], and 90.1 per 1000 at age 85 to 88[2]. Diseases causing dementia have received considerable attention during the last decade, especially after the introduction of the cholinesterase inhibitors, reports that describe the heavy burden on caregivers caused by the dementia diseases, and the enormous socio-economic costs[3]. According to many studies, dementia is underdiagnosed, especially in its early phase[4,5]. There are several reasons why dementia should be diagnosed. Information about the diagnosis may enable the patient and the caregiver to cope better, and several pharmacological and non-pharmacologial treatments are available[6,7]. In Norway, the general view is that patients with mild or very mild degree of dementia (i.e. Mini Mental Status Examination (MMSE) score above 24) should be assessed by a specialist dementia team with expertise in psychiatry, geriatric medicine and neurology[8]. However, because of the large number of demented patients and the sparse number of specialists in Norway it is recommended that persons with moderate or severe dementia (i.e. MMSE below 24) should be assessed and diagnosed by their general practitioners (GPs)[8]. These patients are in need of care either from the family or from the community health service, and the assessment is therefore better done in a community setting.

The Norwegian Centre for Dementia Research which is a centre for research, education and for the initiation of development projects, provides support to GPs and nurses

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to implement new assessment and treatment programmes for persons suffering from dementia [9]. To explore the needs and wishes of GPs in this matter, a postal inquiry among all Norwegian GPs was carried out. The more specific aim of this survey was to examine the assessment of patients with dementia in general practice, and to get the GPs' views on necessary changes for improving the diagnostic work-up of dementia in primary health care.

Methods

The study was carried out by means of a postal questionnaire, with seven questions with preformed answer alternatives and one open-ended question. The questionnaire was developed from published literature and from our own experience from teaching GPs about dementia for many years. The questionnaire was sent to all GPs in Norway, (3604), in February 1999. After two reminders, 1975 (55%) forms had been returned. Of them, 128 were discarded due to incompleteness, mainly because the GP did not have elderly patients in his/her practices. Thus, data from 1847 GPs (51%) were available for analysis.

Results

Table 1 outlines the main characteristics of the responders, compared with the total sample of general practitioners in Norway. As can be seen from the table, a little more than half of the GPs are specialist in general practice which implies that they have to attend different courses and work in hospital at regular intervals. This certificate has to be renewed every five years. In Norway consultants in nursing homes are mostly GPs working part-time, with an approximately weekly work-load of 2-6 hours. In the present survey 39% of the responders were consultants.

Table 2 shows the content of the assessment carried out by Norwegian GPs when they suspect cognitive impairment or dementia in their patients. A physical examination was always carried out by 71% of the GPs as part of the diagnostic work-up. About 55% of the GPs always performed a clinical interview to assess the patient's cognitive function, but only 23% assessed cognition by means of a test, such as the Mini Mental Status Examination. One of three doctors interviewed the caregiver to collect information of the start, course and type of symptoms. The patient's ability to perform activities of daily living (ADL) was assessed by 40% of the GPs.

To fulfil the definition of dementia according to ICD-10 a decline in memory and two other cognitive domains, in ADL functioning and changed behaviour should be present. All symptoms should have been present for at least six months. To document the presence, duration and degree of the symptoms a family member or a friend of the patient should be interviewed. None of the GPs answered that in every case they would carry out a complete diagnostic work-up consisting of a physical examination, a clinical interview/formal testing of cognition, an assessment of ADL, an assessment of behaviour and an interview with a family member.

Table analyses show that female doctors, doctors less than 45 years of age and GPs working as consultants in nursing homes more often used cognitive tests in the diagnostic work-up ($p<0.05$, 0.005 and 0.005 respectively, after adjustment for multiple significance testing). Specialists in general practice had significantly more contact with family caregivers than non-specialists. They more often interviewed the caregivers during the diagnostic work-up, assessed their situation and gave information of the results from the diagnostic workup ($p < 0.005$).

Concern raised by the family was the most frequent reason (50%) for GPs to carry out a diagnostic work-up. Problems identified during a consultation were the second most frequent trigger (21%), and 193 GPs mentioned patient self-report about reduced memory. Medication problems were reported from 160 GPs. Only 13 GPs reported that the mandatory health check for drivers aged 70 years and older was used as a trigger for dementia assessment.

More than 85% of the responders agreed that GPs should play a central role in the diagnostic assessment of patients with suspected dementia in primary health care. To the question "are the conditions for this work-up sufficient today", the majority (51%) answered yes, 27% no, 9% did not know. Thirteen percentages did not answer this question. The most frequent reasons for answering no were time constraints, lack of financial reimbursement, and lack of knowledge how to carry out a diagnostic assessment.

Table 1. Characteristics of the GPs who responded (n=1847) and of the general population of general practitioners in Norway (n=3604).

	n=1847	n=3604
Age of the GPs, mean	45.3 years	44.6 years
Gender, % males	75.0%	72.7%
Specialisation, % specialists in general practice	65.9%	53.3%
Consultants in nursing homes	38.7%	*

* Data not available

Table 2. The content of the assessment used in the diagnostic work-up of dementia in GPs' practice (n=1847).

Examinations	Always	Often	Occasionally	Never/seldom
Patient examination:				
-Physical examination	70.6%	24.4%	4.6%	0.3%
-Clinical interview	55.5%	36.7%	7.5%	0.7%
-Use of cognitive tests	23.3%	33.4%	28.8%	14.5%
-Assessment of activities of daily living	40.0%	45.6%	13.4%	1.0%
-Assessment of behaviour/depression	47.0%	46.0%	6.8%	0.2%
Interview with the caregiver	30.7%	49.6%	18.4%	1.3%
Assessment of the caregivers' situation	19.5%	48.0%	29.7%	2.8%
Information to the patient about the diagnosis	19.6%	42.7%	31.8%	5.9%
Information to the caregiver	32.4%	51.4%	15.3%	0.9%
Contact with the home care services				
- regarding assessment	13.2%	44.9%	34.9%	7.0%
- regarding treatment	20.5%	53.4%	22.5%	3.6%

Table 3. The GPs' opinion of an optimal diagnostic work-up and the use of a multidisciplinary team in primary health care, percentages in parentheses (n=1847).

	GP	Nurse	Occupational therapist	Not necessary
Physical examination	1835 (99.2)	36(1.9)	16 (0.9)	0
Assessment of				
… cognitive function	1580 (86.1)	799 (43.5)	273 (14.9)	1
… activities of daily living	404 (21.9)	1340 (72.7)	1280 (69.5)	1
… behaviour	1173 (64.5)	1474 (81.0)	425 (23.4)	3
… depression / anxiety	1756 (95.4)	765 (41.6)	69 (3.7)	0
… the caregiver's situation	1050 (57.3)	1605 (87.5)	373 (20.3)	7
Clinical interview with the caregiver	1661 (90.5)	1252 (68.2)	316 (17.2)	0
Regular follow-ups	1284 (70.0)	1520 (82.0)	408 (22.3)	7

Table 4. The GPs' suggestions of how to improve assessment and care of patients with dementia in primary health care (n=1484).

	Very important/Important
Improve collaboration in primary health care	73.8%
Increase own practical competence (interview, practical testing)	73.8%
Increase own theoretical knowledge	73.2%
Wider availability of specialist services	63.4%
Extra payments for assessing patients with dementia	51.7%
Special GPs should have responsibility for the diagnostic work-up	13.5%

The GPs were asked what the assessment of dementia in general practice should include, and which professions of the multidisciplinary team in primary health care should take part. The answers are shown in table 3. Except for the assessment of the patient's ability to carry out activities of daily living, the GPs did not want assistance from occupational therapists. In most cases they prefer to carry out the diagnostic work-up themselves and/or in co-operation with district nurses.

Seventy-nine percent of the GPs answered that changes in general practice are necessary to improve the diagnostic work-up in primary care. A table with suggestions for improvements were included in the questionnaire. Improved collaboration between various health professionals working in primary health care (74%), better theoretical and practical knowledge (73%) and wider availability of specialist services (63%) were suggested to improve the work-up of dementia in primary health care (table 4).

Discussion

A well-known problem with postal questionnaires to GPs is the low response rate, which is also a limitation of the present study. The non-responder group may have a different way of handling patients with suspected dementia. In any case, the results demonstrate a lack of uniformity among the GPs regarding the diagnostic work-up of dementia.

Rather few of the GPs in this study interview caregivers on a regular basis when assessing a patient suspected of dementia. To our knowledge, very few studies have focused on this aspect of the dementia assessment. A study made by Brodaty had similar findings to our study [10]. In it 30% of the Australian GPs interviewed the carer. However, in a study among 301 GPs in France, Ledésert found that over 90% sought information from carers [11]. Many of the patients lack insight into their disease and cannot give reliable information either about the start and duration of cognitive impairment or about the behavioural changes. Therefore, an interview with a person who knows the patient well must be carried out. Such an interview should, in our view, be mandatory when making a diagnosis of dementia. Information from a caregiver is essential to make a diagnosis of dementia according to International Classification of Disorders (ICD-10; WHO, 1992, [12]) or Diagnostic and Statistical Manual of Mental Disorders (DSM-IV; APA, 1994, [13]) because information of the patient' ability to carry out the activities of daily living is crucial. Norwegian GPs as well as GPs in other countries normally use neither ICD-10 nor DSM-IV. They are obliged to use the International classification of primary care (ICPC; WONCA, 1998, [14]) diagnostic system, which lacks any description of dementia. This might explain why Norwegian GPs seldom collect information from a family informant. However, as shown both in this and in previous studies, reports from family members most frequently triggered a diagnostic work-up [15]. Therefore, the GPs should have access to interview the family caregiver .

In our view, interviewing the family caregiver is one of the most important issues when assessing dementia, not only for diagnostic purposes, but also to assess their situation as the carer. Numerous studies have documented the burden and the psychological and physical morbidity associated with the role of being a caregiver to a patient with dementia [16]. Large randomised controlled trials have shown that caregiver intervention programmes can reduce psychological morbidity in caregivers, delay institutionalisation and save the community costs associated with nursing home care [7,17]. Therefore, to reduce caregiver strain as much as possible, the assessment of a patient with suspected dementia should be initiated early in the disease and the family caregivers should be involved from the beginning.

Rather few of the GPs report that they make use of formal cognitive tests routinely when assessing a patient suspected of dementia. The rather infrequent use of short dementia tests has been reported in previous studies [11], and is in contrast to existing recommendations and guidelines [8, 18, 19]. Physical examination is in most cases carried out, which is necessary for instance to rule out diseases which can mimic dementia (i.e. delirium), but physical examination cannot replace an interview with the caregiver

or the use of a formal cognitive test. As suggested in a previous study, the GPs probably do not have routines for assessing patients with suspected dementia, and are less experienced in carrying out family interviews and cognitive testing compared to physical examinations [20].

As shown in previous studies, few GPs always inform the patient and caregivers about the dementia diagnosis [21]. This may reflect the attitude that any patient with dementia does not understand this kind of information or the fact that the diagnosis probably is made late in the disease. This may change in the near future because of the introduction of new anti-dementia drugs, the cholinesterase inhibitors, which probably should be introduced early in the course of the disease. Perhaps more patients with dementia in an early phase will see GPs for diagnostic evaluation and treatment.

We found it a little surprising that the vast majority of the GPs in this study view themselves as having the major role in the assessment and follow-up of demented patients. However, only half of them considered that they had the opportunity to play this role. The GPs in this study claimed that the main reasons for insufficient assessment of demented patients are lack of financial reimbursement, lack of knowledge about dementia and lack of time. In an editorial in the Journal of the Norwegian Medical Association called "The filled-up bucket in general practice" this last-mentioned problem is discussed. The author writes: "a general practitioner cannot do unlimited number of tasks in his patient population" [22]. In another paper, McIntosh writes that the GP input in the area of dementia may be minimal, but remains essential [23].

Conclusion

According to the findings from this study, Norwegian GPs wish to play a major role in the assessment of patients with suspected dementia. More than 70% of the GPs suggested that assessment of dementia in primary health care could be improved by increasing the doctors' theoretical knowledge and practical competence. We support this view and believe that educational programmes should focus on fields that GPs are not normally trained in, such as formal cognitive testing and interviewing carers.

In addition to practical training and increased theoretical knowledge of dementia, we believe that development of a diagnostic programme, based on the diagnostic criteria according to ICD-10 or DSM-IV, is essential. Due to the GPs' work load, the programme must be simple and adapted to their squeezed situation. To improve the quality of the assessment the authors of this study have in co-operation with GPs with an interest in dementia developed a simple diagnostic programme, one part for GPs and another for nurses in primary health care. The GPs part is feasible in a busy general practice and is now integrated with the various IT based patient record systems used in Norway.

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Further Evidence in Support of the Presence of Behavioral Syndromes in Alzheimer's Disease

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Abstract

In an independent group of AD patients we replicated previous findings showing that behavioral disturbances can be grouped into separate behavioral syndromes. The new group of 74 AD patients was less cognitively impaired than the historical group (MMSE 21 SD 2 *vs.* 13 SD 7 $p<0.01$). Behavioral disturbances were assessed with the Neuropsychiatric Inventory. Behavioral syndromes were identified with factor analysis. Both in the present and in the historical groups four factors solution (mood, frontal, psychosis and agitation) accounted for similar proportions of explained variance (75 *vs.* 74%). The prevalence of patients with no syndrome (62 *vs.* 55%), any 1 or more (38 *vs.* 45%), and any two or more syndromes (18 *vs.* 27%) was similar in the two groups ($p>0.37$). Most patients with 1 or more syndromes had isolated syndromes in both groups (82 *vs.* 73%, $p>0.87$). The results support the notion that behavioral syndromes can be identified in AD.

Keywords: behavioral disturbances, Alzheimer's disease, disease severity, factor analysis.

Background

Behavioral disturbances are common in Alzheimer's disease (AD) and cause functional disability and poor patient and caregivers' quality of life. However, the biological determinants are still unclear. This might be due to the fact that most studies having focused on individual disturbances (such as depression or agitation)[1-3]. On the contrary, clinical observation and research suggest that some behavioral disturbances tend to occur together in the same patient leading to hypothesize that studying these syndromes rather than individual disturbances might help identify biological determinants. However, which individual disturbances define each syndrome still needs to be clarified.

In a previous study on AD patients with wide range of disease severity we found that individual disturbances occurring together in a given patient could be grouped into three syndromes: mood (characterized by anxiety and depression), psychosis (agitation, hallucinations, delusions and irritability) and frontal syndrome (disinhibition and euphoria). Consistently with the hypothesis of specific biological determinants, the syndromes tended to occur isolated in a given patient: patients with a given syndrome tended not to show either of the other two. However these findings were based on a single group of patients of heterogeneous severity and replicating the findings is necessary.

Aim of this study was to confirm these findings in an independent group of AD patients of homogeneous dementia severity. It can be hypothesized that if the syndromes have a specific biological basis, 1) similar syndromes should be found 2) the syndromes should present isolated in individual patients and, 3) the prevalence of the syndromes in the two groups should be consistent with the disease severity of the two groups. Factor analysis will be used, factors being the mathematical representation of syndromes.

Material and methods

The historical group[4] was constituted by 162 consecutive patients with probable AD, of any severity who come to observation of the Alzheimer's Unit of the IRCCS S. Giovanni di Dio, from June 1995 to December 1996. The subjects of the new group were 74 mild (Mini Mental State Examination of 18 or higher)[5] AD patients consecutively admitted to the same Unit who were enrolled in a prospective study on the natural history of dementia. After a complete description of the study

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to the subjects, written informed consent was obtained. Comprehensive description of the clinical and instrumental assessment procedures and the clinical features of the two groups have been provided elsewhere[4,6].

Behavioral disturbances (anxiety, delusions, hallucinations, depression, irritability, disinhibition, euphoria and agitation) were assessed with the Neuropsychiatric Inventory (NPI)[7]. For each disturbance the frequency (score 0 to 4) and the severity (score 0 to 3) were evaluated to compute a global score (frequency x severity, 0 to 12).

The factor analysis of the previous study was re-run on the new as well as on the historical patients groups. The syndromes and their constituting behavioral disturbances were identified with principal component analysis, with orthogonal rotation of the factors, factor scores being the mathematical representation of syndromes. As in the historical analysis 3, 4 and 5 factor solutions were tried in both groups, and the solution with the most easily interpretable factors was retained. A syndrome was considered present in a given patient if the standardized factor score was 1 or higher. The solution was confirmed with oblique rotation (a detailed description of the statistical analysis can be found elsewhere)[4].

The study has been reviewed and approved by the local ethics committee.

Results

Table 1 shows that age, gender and education were similar in the two groups. The severity of dementia of the historical group was on average moderate, with a wide range. The severity in the present group was mild, and the range was narrower.

The four-factor solution (table 2) was the most interpretable and accounted for 75% of the variance in the present and 74% in the historical AD group. Overall, the variance explained by each factor was similar in the two groups. The largest difference was in the psychosis factor, accounting for 14% of the variance in the present and 20% in the historical group. Symptoms characterizing the factors had similar factor loadings in the two groups. Differences were small (lower that .20) in 7/11 symptoms and between 0.20 and 0.40 in 2/11 (delusions in the psychosis and disinhibition in the frontal factor). Only two symptoms in the historical group (anxiety in the psychosis and disinhibition in the agitation factor) failed to reach the criterion to qualify as significant contributors to the factors.

Overall, the historical and the present AD groups were characterized by similar prevalence of each syndrome (table 3). The prevalence of patients with none of the syndromes was non- significantly higher in the present group, while among those with one or more syndromes, those with two or more were more frequent in the historical group, albeit non-significantly. Of those with only 1 syndrome, mood and agitation were the most frequent, and frontal and psychosis the least frequent in both groups. However, the former two syndromes tended to be more frequent, and the latter was less frequent in the present than in the historical group, once more failing to achieve statistical significance.

The last part of the analysis was addressed to identify the demographic and the clinical correlates of the sub-groups. The subgroups were not different for gender, education or age at onset neither in the present or in the historical groups. However, in the present group those patients with "pure" agitation syndrome were significantly older with respect to the others pulled together (80.6 SD 5.7 *vs.* 75.5 SD 8.7 $p<0.05$). In the historical group the mean age of the agitation subgroup was higher, though it failed to reach statistical significance (79.2 SD 7.1 *vs.* 76.2 SD 8.7 $p=0.12$).

Discussion

The results of this study have successfully replicated previous findings in an independent group of AD patients. In fact, according to the hypothesis of separate behavioral syndromes, factor analysis defined four syndromes and each syndrome was characterized by a similar pattern of symptoms with similar factor loadings both in the present and in the historical group. The syndromes have a defined profile. Agitation is characterized mainly by agitation and irritability, mood is consistently marked by depression and anxiety, hallucinations are the hallmark of the psychosis and euphoria of the frontal syndrome. Few patients had mixed syndromes indicating that it is possible to identify "pure" behavioral profiles. Moreover, the relative prevalence of each syndrome in the two groups was similar being mood and agitation syndromes the most prevalent while frontal and psychosis the less frequent in both groups.

The prevalence of the syndromes had a similar pattern although the groups differed for disease severity. The difference of disease severity between the two groups had a minor effect on the frequency of "pure" factors, reflecting proportions of mood and agitation syndromes in the present and of frontal and psychosis in the historical groups. However the factor solution and the factor loadings were similar. The above mentioned differences between the two groups were consistent with the notion that depressive symptoms, anxiety and delusions (in particular of paranoid type) are more likely to occur in the earlier stages of disease[8-11], and become progressively rarer when the disease progresses. Similarly, the lower frequency of the psychosis syndrome is in agreement with observations indicating that hallucinations and frontal impairment are more likely to occur in the later stages.[12]

However the small size of the groups with isolated syndromes (n=23) prevented differences to achieve statistical significance.

Table 1. Sociodemographic and clinical features of the historical (n=162) and present (n=74) groups of AD patients.

	Historical n=162	Present n=74	*p**
Age [range]	76 (9) [55-96]	76 (8) [57-90]	N.S.
Gender, female	120 (74%)	56 (76%)	N.S.
Education, years [range]	5.7 (3.1) [2-19]	6.0 (3.2) [2-18]	N.S.
Mini Mental State Examination [range]	13 (7) [0-24]	21 (2) [18-27]	0.005

Values denote mean (standard deviation) or number (%).
* *p* denotes significance on t-test for independent samples or on Chi – square.

Table 2. Factor analysis of the NeuroPsychiatry Inventory subscales in the historical and present group of AD patients.

Factor	% of variance explained by each factor		Symptoms*	Factor loading	
	Historical	Present		Historical	Present
Agitation	30	29	Agitation Irritability Delusions Disinhibition	0.84 0.84 0.41 —-	0.84 0.78 0.60 0.60
Mood	13	18	Depression Anxiety	0.81 0.79	0. 70 0.89
Psychosis	*20*	*14*	Hallucinations Delusions Anxiety	0.91 0.79 —-	0.91 0.40 0.43
Frontal	12	14	Euphoria Disinhibition	0. 74 0. 87	0.93 0.53

* Only symptoms with factor loading > 0.30 are shown.

Table 3. Prevalence of the behavioral syndromes (agitation, psychosis, frontal, mood) isolated or in combination in the historical and present group of AD patients.

	Historical	Present	*p**
No syndrome	89 (55%)	46 (62%)	0.37
1 or more	73 (45%)	28 (38%)	
2 or more	20 (27%)	5 (18%)	0.46
Only 1	53 (73%)	23 (82%)	
Mood	17 (23%)	9 (32%)	0.28
Frontal	11 (15%)	1 (4%)	
Psychosis	8 (11%)	2 (7%)	
Agitation	17 (23%)	11 (39%)	

A couple of caveats of this study should be noted. First, the new factor solution identified four instead of the three syndromes of the previous study. Those symptoms that in the previous study were more likely to be grouped in the psychosis syndrome, in the present work identify two different syndromes: psychosis and agitation. This difference might be due to the equivocal meaning of the delusion sub-scale of NPI, addressing symptoms that might have different pathogenesis, such as paranoid delusions, which tend to occur early and might load on the agitation syndrome, as well as misidentifications which tend to occur late in the disease course and might load on the psychosis syndrome. Second, in the present group we have been unable to confirm the clinical correlates of the syndromes found in the previous study. In fact while in the previous work the psychosis group tended to be older, to have later onset of dementia, poor cognition examination, and faster progression of cognitive impairment, in the present study no significant differences were found across the four groups except for the agitation syndrome whose patients were more likely to be older both in the present and in the historical group.

Further studies aimed to detect the neurobiological and neurochemical substrate of each syndrome are needed in order to devise specific pharmacological treatments targeted to the syndrome rather than to individual symptoms.

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