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ORIGINAL ARTICLE

Pleasant physical exercise program for prevention of cognitive decline in community-dwelling elderly with subjective memory complaints

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Aim: Japan is one of the most rapidly aging societies in the world. Measures to prevent dementia are urgently required in Japan, although such strategies have not yet been established. This study investigated the effectiveness of a pleasant physical exercise intervention on the prevention of cognitive decline in community-dwelling elderly participants with subjective memory complaints. In this intervention, a pleasant atmosphere was emphasized to enhance the participants' motivation.

Method: We administered a 12-week intervention program consisting of pleasant physical exercise. This program for the prevention of cognitive decline was carried out as a service of Maebashi city. The service targeted elderly residents aged 65 years and older who had subjective memory complaints. After a control period of 12 weeks, 42 participants, aged between 65–86 years, received intervention once a week at community centers. Participants carried out group exercise, and were encouraged to perform home exercise and walking during the intervention period. The program was carried out by co-medical professional staff, with the help of senior citizen volunteers.

Results: A total of 30 participants were included in the analysis. There was significant improvement on the Wechsler digit symbol substitution test ($P = 0.01$).

Conclusion: Participants with subjective memory complaints who continued the pleasant physical exercise programs for 12 weeks showed improvement in some aspects of cognitive function. Participation of senior citizen volunteers enabled smooth implementation of the program, and alleviated the burden on the professional staff. The pleasant physical exercise intervention described in the present study could be regarded as a community-led intervention to prevent cognitive decline. **Geriatr Gerontol Int 2012; ••: ••–••.**

Keywords: community-dwelling elderly, physical exercise intervention, senior citizen volunteer, service for prevention of cognitive decline, subjective memory complaints.

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Introduction

It is estimated that 24.3 million people have dementia worldwide, with 4.6 million new cases every year.[1] Japan is one of the most rapidly aging societies in the world, and the number of demented elderly people who require nursing care is predicted to be 2.5 million in 2015 and 3.2 million in 2025.[2] Measures to prevent dementia are urgently required in Japan, although such strategies have not yet been established.

Physical exercise intervention for individuals with subjective memory complaints (SMC) is expected to be one of the efficient strategies to reduce the risk of cognitive decline. Several studies have suggested that SMC are associated with increased risk of dementia, even in persons with normal cognitive function.[3–5] A meta-analysis focused on older adults with dementia and related cognitive impairments suggested that physical exercise increases fitness, physical function, cognitive function and positive behavior.[6] In non-demented subjects, the results of a recent meta-analysis showed that subjects who carried out physical activity had a significantly reduced risk of cognitive decline.[7] A randomized controlled trial in older adults with SMC showed that physical activity programs were associated with an improvement in cognition.[8]

Physical activities in a pleasant atmosphere can be more effective for the prevention of cognitive decline. It has been proven in an animal study that exercise in enriched environments has a suppressive effect on the accumulation of amyloid β protein.[9] We have proposed the efficacy of intervention carried out in a pleasant atmosphere with an emphasis on communication (brain-activating rehabilitation).[10] Therefore, it could be meaningful to facilitate a pleasant atmosphere, and form a group where participants enjoyed mutual communication.

In Japan, public concern about care prevention has been growing since the Long-Term Care Insurance system was revised in 2008. Many municipalities have already started services for preventing cognitive decline. The services focus on maintaining and/or improving the cognitive functions of those who do not require care at present. However, the effectiveness of these services is currently insufficient. Furthermore, it remains necessary to prove the effectiveness of such services in preventing cognitive decline, if the services are to be provided as a public service.

We carried out a pleasant physical exercise intervention, which was conducted as a service of Maebashi city, in elderly with SMC. The programs were administered by co-medical professional staff along with senior citizen volunteers. The present study investigated the effectiveness of this service for preventing cognitive decline in elderly residents with SMC.

Method

Participants

The intervention program was carried out for the prevention of cognitive decline as a service of the municipality of Maebashi city in 2010. The service targeted elderly subjects aged 65 years and older residing in two districts of Maebashi city. Participants were recruited from these districts by the following methods.

1 Lectures on the prevention of cognitive decline for community residents were held twice.
2 Leaflets were distributed to each household, 1958 in total.
3 Public health nurses and local welfare commissioners visited door-to-door to invite elderly residents to the program.

The Medical Ethics Committee of Gunma University approved this study (21–47), and written informed consent was obtained from all participants.

Initial screening

Participants ($n = 100$) were screened by a questionnaire and medical interview (Fig. 1). They were examined by a clinician specializing in dementia. Those who met the two criteria below were excluded, and 87 participants remained.

1 Diagnosed as having dementia according to the criteria of International Statistical Classification of Diseases and Related Health Problems 10th Revision (ICD-10).
2 Having a medical condition that made them unable to engage in physical activity.

Evaluation

The change in cognitive function was evaluated using Five-cog test, which evaluates the following cognitive domains: attention, memory, visuospatial function, language and reasoning. The Five-cog test consists of five items: (i) "character position referencing task" for evaluating attention; (11) "category cued recall task" for evaluating memory ability; (iii) "clock drawing task" for evaluating visuospatial function; (iv) "animal name listing task" for evaluating language ability; and (vi) "analogy task" for evaluating abstract reasoning ability.[11,12] Participants were also evaluated using the Wechsler digit symbol substitution test (WDSST).

To evaluate the physical function of each participant, grip strength test, one-leg standing duration test, timed up and go test, and 5 m maximum walking times test were carried out.

Participants were required to complete a questionnaire consisting of questions regarding age, sex, education and previous/current medical history. Their

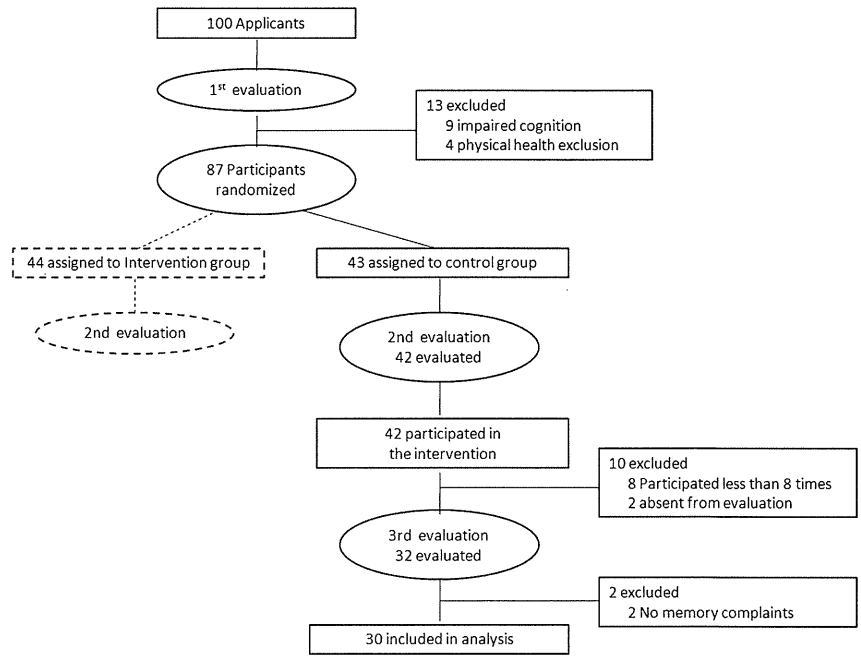

Figure 1 Flow of participants through the study.

subjective health status was evaluated with the question "How is your health in general?" using a rating scale from 1 = excellent to 4 = poor. The level of social support was evaluated with the Lubben Social Network Scale Revised (LSNS-R), which gauges social isolation in older adults by measuring the perceived social support received by family, friends and neighbors.[13] Functional capacity was determined using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC).[14] TMIG-IC is a multidimensional 13-item index of competence comprising three dimensions; that is, instrumental self-maintenance, intellectual activity and social role. The index was designed to measure higher level competence in community-dwelling elderly.[14,15] The Satisfaction in Daily Life (SDL), a simple measurement of subjective quality of life (QOL), was used to evaluate the life satisfaction of participants.[16,17] The SDL consisted of 11 items; that is, physical health, mental health, self-care, gait, housework, house facilities, partner and family relationships, hobby and leisure activities, social intercourse, economic state and social security, and having a job. Each item was rated from 1 = dissatisfied to 5 = satisfied. The 15-item short version of the Geriatric Depression Scale (GDS)[18] was used to evaluate depressive symptoms.

Study design

Evaluation was carried out three times (Fig. 1). After receiving the initial screening and evaluation (first evaluation), 87 eligible participants were randomly divided into two groups of 43 and 44. Participants in the present study ($n = 43$) were allocated to the control group of another randomized controlled trial (RCT), which is still continuing by adding participants in another district of Maebashi city. The participants in the present study received an educational lecture about nutrition during the control period. After the 12-week control period, 42 participants were evaluated for baseline condition (second evaluation). Effects of the intervention were evaluated at the end of the intervention period (third evaluation). At each evaluation, cognitive and physical function tests and questionnaires were administered to participants. Participants were examined by the questionnaire administered in the first evaluation as to whether they had SMC or not on the following two questions: "Are you concerned about forgetfulness?" and "Have you experienced any memory problems over the past 1 month?". Participants were considered to have SMC if they answered yes to both of the questions.

Participants were considered as having amnestic mild cognitive impairment (aMCI) by a clinician specializing in dementia according to the following criteria:[19] reported memory complaint; objective memory impairment for age; essentially preserved general cognitive function; largely intact functional activities; and not demented. They were included in SMC in accordance with a previous research on SMC.[20] Participants without SMC were excluded from analysis. However, they participated in the intervention program, because the intervention was carried out as a community service that should be available to all community dwellers.

Intervention

We administered the 12-week intervention program consisting of pleasant physical activity for 42 participants (Fig. 1). The exercise program includes muscle-stretching exercise in a sitting position (17 items), muscle-strengthening exercise in a sitting position (3 items), muscle-strengthening exercise in a standing position (7 items) and aerobic exercise (3 items). These exercises require comprehensive ability in physical activity consisting of body flexibility, muscle-strength, balance and endurance. The mean duration of the exercise program was 45 min. Participants were encouraged to carry out home exercise based on the exercise program. Walking was recommended to participants as regular exercise. Leisure activities and educational lectures were included in the program in order to motivate participation; for example, cooking, handcraft activity, lectures on physical activities and dental health. Participants received programs once a week in community centers located in the districts where they resided. The program was carried out in two groups of 26 and 16. The program was provided by the following co-medical professional staff: physical therapist, occupational therapist, public health nurse, dietitian and dental hygienist.

Senior citizen volunteers also participated in every program. Maebashi city has promoted participation of the elderly in volunteer activities focusing on long-term care prevention in the community. In the present intervention, 27 senior citizens participated as volunteers. They received training sessions on brain-activating rehabilitation, which emphasized a comfortable atmosphere; empathetic communication with each other; praising each other; having a social role; and errorless learning.[10] They were expected to facilitate communication among the participants and maintain a pleasant atmosphere. In each program, three to seven volunteers participated in assisting the professional staff in carrying out the program.

Statistical analysis

Statistical analysis was carried out using the Japanese version of SPSS, 17th edition (IBM, Armonk, NY, USA). Analyses were carried out with all the participants, and with those having aMCI and those not having aMCI separately. All results were analyzed by repeated-measures analysis of variance (ANOVA) and Bonferroni's correction post hoc test. We regarded $P < 0.05$ as showing significance.

Results

Flow of participants through the study

Figure 1 shows the flow of participants through the study. In the present study, 42 participants underwent the program after the control period of 3 months. The attendance rate during the intervention was 77.4%. A total of 10 were excluded from the analysis; eight attended the program less than eight times and two were absent from the third evaluation. Two participants were considered to not have SMC based on their answers on the questionnaire. Finally, 30 were included in analysis. Table 1 shows the demographic and clinical characteristics of 30 included participants and those of 12 excluded participants.

Analysis of all the participants

In the control period (between the first and second evaluation), there was no significant increase in the scores on cognitive and motor tests, except for the "Cued recall task" on the Five-cog test ($P = 0.001$) (Table 2). In the intervention period (between the second and third evaluation), significant improvement was seen on the "Cued recall task" of the Five-cog test ($P < 0.001$) and WDSST ($P = 0.01$) on the cognitive tests (Table 2). There were no significant changes between

Table 1 Demographic data of the participants

Characteristic	Participants included ($n = 30$)	Participants not included ($n = 12$)	P-value‡
Age† (years)	73.7 ± 5.5	75.0 ± 7.7	0.593
Female, n (%)	26 (86.7)	10 (83.3)	0.561
Years of education†	11.6 ± 3.8	11.4 ± 2.9	0.881
MMSE score†	28.4 ± 1.5	27.3 ± 2.0	0.067
SMC with aMCI, n (%)	7 (23.3)	6 (50.0)	0.095

†Results are expressed as: mean ± standard deviation. ‡Significance was tested using independent Student's t-tests for continuous variables, and χ^2-tests for categorical variables. aMCI, amnestic mild cognitive impairment; MMSE, Mini-Mental State Examination; SMC, subjective memory complaints.

Table 2 Results of the test scores (all participants, $n = 30$)

Scale	Ev. 1† (before Control period)	Ev. 2† (before intervention period)	Ev. 3† (after intervention period)	*F*-value	*P*-value	Ev.1 *vs* 2	Ev.2 *vs.* 3
Five-cog test							
Character position referencing task	21.3 ± 8.3	21.9 ± 9.1	23.7 ± 8.0	4.171	0.020*	1.000	0.107
Cued recall task	14.1 ± 4.9	17.0 ± 5.5	21.0 ± 6.2	53.189	<0.001***	0.001**	<0.001***
Clock drawing task	6.5 ± 1.4	6.8 ± 0.61	6.9 ± 0.4	3.093	0.084	0.490	0.130
Animal name listing task	15.9 ± 4.7	17.6 ± 5.4	19.0 ± 5.4	8.361	0.001**	0.071	0.173
Analogy task	10.1 ± 2.8	10.8 ± 3.1	10.9 ± 2.9	3.788	0.028*	0.046*	1.000
WDSST	52.7 ± 14.8	56.3 ± 14.2	60.4 ± 15.2	10.050	<0.001***	0.192	0.010*
Subjective health status	2.1 ± 0.5	2.2 ± 0.5	2.1 ± 0.4	0.693	0.504	0.791	1.000
LSNS-R	17.3 ± 5.5	16.4 ± 6.1	17.5 ± 5.6	1.154	0.316	0.975	0.253
TMIG-IC	11.9 ± 1.9	12.1 ± 1.5	12.0 ± 1.7	0.931	0.385	0.249	1.000
SDL	20.9 ± 4.2	20.6 ± 4.1	21.1 ± 4.2	0.254	0.776	1.000	1.000
GDS	3.5 ± 2.9	3.3 ± 2.3	2.7 ± 2.4	2.238	0.116	1.000	0.377
Grip strength	24.9 ± 6.2	26.3 ± 5.7	26.0 ± 6.2	4.212	0.028*	0.072	1.000
One-leg standing duration	34.7 ± 20.0	39.5 ± 21.3	39.8 ± 22.5	2.036	0.140	0.247	1.000
Timed up and go test	5.9 ± 0.8	6.2 ± 1.0	6.1 ± 0.9	3.388	0.041*	0.034*	0.613
5 m maximum walking time	2.7 ± 0.4	2.6 ± 0.4	2.6 ± 0.3	0.081	0.922	1.000	1.000

†Results are expressed as: mean ± standard deviation. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. Ev., evaluation; GDS, 15-item short version of the Geriatric Depression Scale; LSNS-R, Lubben Social Network Scale Revised; SDL, The Satisfaction in Daily Life; TMIG-IC, Tokyo Metropolitan Institute of Gerontology Index of Competence; WDSST, Wechsler digit symbol substitution test.

participants on any item on the questionnaire. None of the physical function tests showed significant changes after the intervention.

Subanalysis of participants without aMCI and with aMCI

When seven participants who were considered to have aMCI were excluded, results of subanalyses in 23 participants were similar to those of the analyses in all participants. There were no significant changes shown on analyses of seven participants with aMCI.

Discussion

The present study investigated the effectiveness of pleasant physical exercise intervention, provided as a service for the prevention of cognitive decline in community-dwelling elderly with SMC. Findings were objectively investigated using cognitive, physical, functional, social and behavioral outcome measures. Through the 12-week intervention, participants with SMC showed improvement in some aspects of cognitive function. In the evaluation carried out after the intervention, significant improvement was seen on the "Cued recall task" of the Five-cog test and on WDSST. Concerning the "Cued recall task", the effects of repeated learning could not be ruled out, as significant improvement was also seen on baseline evaluation.

WDSST is the test of attention and executive function. A previous study suggested that WDSST score is the best cognitive measure to detect unsafe drivers with early dementia of the Alzheimer type and non-demented drivers.[21] Improvement shown on WDSST score suggests that participants have increased abilities to carry out activities of daily living that require attention and executive function.

Although the present study program did not include direct cognitive stimulation, participants showed improvement in cognitive tests scores. The efficacy of intervention carried out in a pleasant atmosphere with an emphasis on interactive communication has been proposed.[10] Throughout the program, the staff and the volunteers were expected to facilitate communication among the participants and maintain a pleasant atmosphere. It is possible that physical activity carried out in the pleasant atmosphere and interactive communication enhanced motivation, which led to improvement of cognitive function.[22]

Preventive programs as municipally-sponsored measures against cognitive decline among community-dwelling elderly have not yet been established. In order to provide appropriate and effective services to prevent cognitive decline, determination of participants and the promotion of effective programs are critical issues. The present study targeted people with SMC. The majority of elderly participants report SMC,[23,24] and the presence of SMC is considered to be an important first sign or indicator of imminent dementia.[3–5] Participation of elderly subjects with SMC is recommended, because they need such an intervention, and benefits can likely be obtained.

We administered a 12-week intervention of pleasant physical activity program. It has been suggested that physical activity reduces the risk of cognitive decline among demented and non-demented elderly participants.[6,7] Therefore, physical activity should be one of the preferred programs for preventing cognitive decline. Furthermore, physical activity programs have several advantages; it is labor- and time-saving, and cost effective. It might be a competent program to offer as a community service.

Use of volunteers was emphasized in our intervention. It is important to develop human resources who can continuously attend preventive care activities in the community, as the shortage of professional staff has become obvious in an aging society. Participation of volunteers enabled smooth implementation of the program, and alleviated the burden on professional staff. Senior volunteers who joined in the intervention played important roles in facilitating a pleasant atmosphere and smooth communication among participants. Involvement of senior citizen volunteers could be effective for a community-based intervention program for the prevention of cognitive decline.

The present study had several limitations. The number of participants was small; the present study first targeted 42 participants, and 30 participants were finally included in analysis as a result of the 77.4% attendance rate of the intervention. The period of the intervention was relatively short; the intervention was carried out for just 3 months. These factors might limit the ability to generalize the results of the study.

In conclusion, participants with subjective memory complaints who continued the pleasant physical exercise programs for 12 weeks showed improvement in some aspects of cognitive function. Participation of senior citizen volunteers enabled smooth implementation of the program, and alleviated the burden on the professional staff. Thus, the present study showed a community-led intervention for care prevention.

Acknowledgment

Author contributions: Tadahiko Kamegaya prepared the manuscript mainly, the intervention program described in the present study was provided by a physical therapist, an occupational therapist, public health nurses, a dietitian, and dental hygienists of Long-Term-Care Prevention Team of Maebashi City. Tetsuya Yamagami and Yohko Maki contributed to the preparation of the manuscript. Tomoharu Yamaguchi and

Tatsuhiko Murai contributed to the collection of data. Haruyasu Yamaguchi made the final approval of the manuscript to be published.

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Disclosure statement

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Effects of Intervention Using a Community-Based Walking Program for Prevention of Mental Decline: A Randomized Controlled Trial

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OBJECTIVES: To evaluate the efficacy of a municipality-led walking program under the Japanese public Long-Term Care Insurance Act to prevent mental decline.

DESIGN: Randomized controlled trial.

SETTING: The city of Takasaki.

PARTICIPANTS: One hundred fifty community members aged 72.0 ± 4.0 were randomly divided into intervention (n = 75) and control (n = 75) groups.

INTERVENTION: A walking program was conducted once a week for 90 minutes for 3 months. The program encouraged participants to walk on a regular basis and to increase their steps per day gradually. The intervention was conducted in small groups of approximately six, so combined benefits of exercise and social interaction were expected.

MEASUREMENTS: Cognitive function was evaluated focusing on nine tests in five domains: memory, executive function, word fluency, visuospatial abilities, and sustained attention. Quality of life (QOL), depressive state, functional capacity, range of activities, and social network were assessed using questionnaires, and motor function was evaluated.

RESULTS: Significant differences between the intervention and control groups were shown in word fluency related to frontal lobe function ($F(1, 128) = 6.833$, $P = .01$), QOL ($F(1,128) = 9.751$, $P = .002$), functional capacity including social interaction ($F(1,128) = 13.055$, $P < .001$), and motor function (Timed Up and Go Test: $F(1,127) = 10.117$, $P = .002$). No significant differences were observed in other cognitive tests.

CONCLUSION: Walking programs may provide benefits in some aspects of cognition, QOL, and functional capacity including social interaction in elderly community members. This study could serve as the basis for implementation of a community-based intervention to prevent mental decline. **J Am Geriatr Soc 60:505–510, 2012.**

Key words: prevention of mental decline; social interaction; dementia; Alzheimer's disease; mild cognitive impairment

The Japanese public Long-Term Care Insurance Act was launched in April 2000 to respond to the growing elderly population. The revision of the act in 2008 led to a greater emphasis on preventive long-term care, and municipalities are expected to play leading roles in building the platform and network for preventive activities.[1]

The Preventive Long-Term Care program was initiated under the leadership of the Ministry of Health, Labor, and Welfare in Japan, where municipality-led preventive interventions have been encouraged in agreement with the concept of community-based rehabilitation,[2] but prevention against mental decline remains a concern, so three areas in Japan (Tokyo, Ohbu, and Takasaki) were selected as model areas to evaluate the efficacy of a community-based program for prevention of mental decline. An intervention program was conducted in Takasaki: the Takasaki Project.

In the Takasaki Project, a walking program was chosen for intervention. Previous studies have reported that regular exercise is beneficial for lowering the risk of mental decline in elderly individuals,[3–5] and the efficacy of

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walking for preventing mental decline has been reported.[6,7] Another merit of walking is its low cost; effective prevention strategies would also have public health implications by reducing economic and social burdens.

The program adopted in this intervention encouraged participants to acquire a walking habit by gradually increasing their walking steps in a group setting. Thus, combined benefits of exercise and social interaction could be expected. Social isolation is associated with greater risk of mental decline,[8,9] whereas a rich social network and interaction may protect against mental decline.[10,11]

Based on the intervention described above, the present randomized controlled trial was designed to test whether a walking program was effective in preventing mental decline in elderly individuals without dementia.

METHODS

Participants

The Takasaki Project was conducted between September and November 2010. The flow of participants is shown in Figure 1. As the first step, participants were screened using a questionnaire. The questionnaire consisted of 25 self-completed items, including three items concerning mental decline: "Have others indicated that you may have memory problems, such as 'you often ask the same things repeatedly'?" "Do you need to look up commonly used telephone numbers?" and "Do you sometimes fail to remember the date?" The questionnaire was mailed to inhabitants aged 65 and older; 2,387 residents younger than 80 answered yes to at least one of the three items, and these respondents were regarded to be at high risk of mental decline. Leaflets describing the Takasaki Project were mailed to them, and 153 agreed to participate. An additional 13 participants were also recruited at a municipal center for elderly adults. One hundred sixty-six individuals attended information meetings, and written informed consent was obtained from 162. At the initial baseline assessment, each volunteer completed the Mini-Mental State Examination (MMSE) and a medical interview with a specialist in dementia medicine. During the interview, 12 volunteers were excluded: five meeting *International Classification of Diseases, Tenth Revision*, criteria for the diagnosis of dementia, five aged 80 and older (those who reached 80 after the 25-item self-check questionnaire was mailed), and two with chronic illness. Therefore, 150 volunteers were eligible for randomization as participants. The ethics board of Gunma University School of Health Sciences approved all procedures (No. 21–47).

Assessment

Cognitive Tests

The major outcome variable was change in cognitive function. Cognitive function comprises various components; the Cognitive and Emotional Health Project in the United States focuses on five domains: learning and memory, executive function abilities (e.g., concept formation and abstract thought), language, visuospatial abilities, and sustained attention (ability to focus and perform a simple task).[12] The 5-Cog test[13] consists of five tests covering the following domains: learning and memory (category cued delayed recall test consisting of 32 words in eight

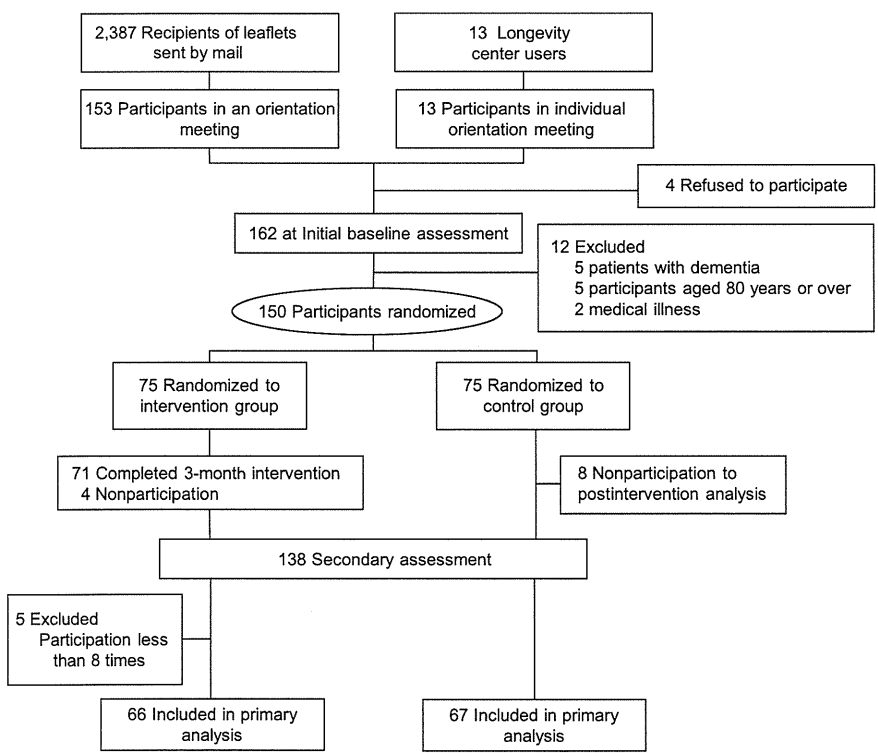

Figure 1. Flow of participants from the time of recruitment through study completion at 3 months.

categories), executive function abilities (dual-task test that requires alternating attention, abstract reasoning test similar to the Wechsler Adult Intelligence Scale-III (WAIS-III)), language (a categorical word fluency test of "animals" completed in two minutes), and visuospatial abilities (a Clock Drawing test to draw clock hands showing the time at "ten after eleven"). Mean scores ± standard deviations (SDs) in participants aged 65 to 80 (n = 800) were as follows: delayed recall test, 12.0 ± 5.8; dual-task test, 20.1 ± 9.1; abstract reasoning test, 10.8 ± 4.3; word fluency test, 13.9 ± 6.0; and Clock Drawing test, 6.7 ± 1.4. The 5-Cog is intended to be conducted in a group setting with a maximum of 50 individuals. The test set is distributed with a 35-minute-long instruction DVD so that the instructions are identical every time. Sustained attention was measured using the Digit-Symbol Substitution Test (DSST), a subset of WAIS-III, and the Yamaguchi Kanji-Symbol Substitution Test (YKSST).[14] The YKSST was developed for Japanese elderly individuals as an adaptation of the DSST; Japanese characters, kanji, were used instead of numbers, as in DSST, because elderly adults in Japan are more familiar with kanji. Mean YKSST scores were 46.9 ± 10.9 in individuals aged 65 to 79 (n = 170). The Trail-Making Test (TMT) was also administered to evaluate executive function abilities. Higher 5-Cog, DSST, and YKSST scores and lower TMT scores indicate better performance.

Questionnaires on Quality of Life, Mood, Functional Capacity, Range of Activity, and Social Network

Participants were required to complete the self-assessment questionnaires. Quality of life (QOL) was measured using a questionnaire on satisfaction in daily life (SDL).[15] Depressive state was evaluated using the Geriatric Depression Scale (GDS).[16] Functional capacity for independent living was assessed using the Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC).[17] The TMIG-IC was designed to measure higher-level functional capacities in community-dwelling elderly individuals and consists of 13 items divided into three subscales: instrumental self-maintenance, intellectual activity, and social role.

The range of activity was measured using the Life-Space Assessment (LSA), which assesses how far and how often a person moves, ranging from moving around the bedroom only to traveling out of the person's town.[18] Social network size was assessed using the Japanese version of the abbreviated Lubben Social Network Scale (Lubben).[19] Higher QOL, TMIG-IC, LSA, and Lubben scores and lower GDS scores indicate better performance.

Assessment of Motor Function

Four tests were conducted: grip force to assess muscle strength, balance time on one foot (60 seconds as cutoff time), Timed Up and Go Test (TUG), and maximum walking speed for 5 meters. Improvement is reflected by an increase in grip force and balance, and by a decrease in TUG and walking speed for 5 meters. In addition to the outcome measures above, average steps per day for 7 days were measured to evaluate the direct effect of the intervention program. All participants wore a pedometer (EX-500; Yamasa Tokei Co. Ltd., Tokyo, Japan) to record the total number of steps walked in a day. Pedometers were sealed so that participants could not see the counters. The effect was assessed by comparing the average steps for 7 days just before and after the intervention.

Randomization

Randomization was conducted at the end of the initial baseline assessment; 150 participants were randomly allocated to the intervention group or control group. Research staff undertaking cognitive assessment, physical assessment, and intervention were separated.

Intervention

The intervention was conducted using the Tokyo Metropolitan Institute of Gerontology method. The program aimed to facilitate walking habits. The 90-minute intervention program was conducted once a week for 12 weeks and consisted of a 30-minute exercise period and 60-minute group work with five to eight people. Each participant was required to set a clear short-term goal on a weekly basis in order to achieve long-term goals. They were required to record their steps every day using a pedometer (during the intervention period, the pedometer was not sealed) and to write a self-assessment of daily activities. In addition to daily walking, participants planned and executed walking events (excursion) with other group members during the intervention period.

The Facilitators

Registered physical trainers or health nurses working at hospitals or healthcare providers commissioned by the local government of Takasaki City conducted the intervention program. They received lectures before the intervention, and they were supervised so that the program was administered appropriately. They were required to behave as facilitators to motivate participants, to maintain smooth communication, and to create a comfortable atmosphere.

Control Group

Participants in this group received educational lectures on food, nutrition, and oral care that were not directly related to the prevention of mental decline.

Analysis of the Data

The data were analyzed using the Japanese version of SPSS for Windows version 19.0 (IBM Corporation, New York). For initial baseline comparison between the intervention and control groups, two-sample *t*-tests were conducted for randomization; there was no significant difference between the two groups for any outcome measure. Participants who underwent the initial baseline and postintervention assessments were included in the final analysis; participants who dropped out and those who were present at the intervention fewer than eight of 12 times were excluded from the analysis. Repeated-measures analysis of covariance, with covariates of age, sex, and years of education, was used to analyze the completed cases. The interaction was examined to assess the differential effect between the intervention

and control groups, and post hoc analysis of within-subject analysis was conducted using Bonferroni correction. Nine cognitive tests, five questionnaires, and four tests of motor function that were independent of each other were conducted. Multiple corrections were not done among these independent measures. Concerning the measures where significant interaction was shown, intention-to-treat analysis was also conducted; five participants who attended fewer than eight times were included in the intention-to-treat analysis.

RESULTS

Demographic data of the participants are shown in Table 1. The attendance rate during the intervention was 87.5%. The analysis was conducted with 66 participants in the intervention group and 67 in the control group (Figure 1).

Direct Effect of Walking Program

The intervention group had a significantly greater increase in average number of steps taken over 7 days from the pre- to the postintervention period than the control group ($F(1,123) = 7.184$, $P = .008$; intervention group, preintervention 5,621 ± 2,494 steps per day, postintervention 7,044 ± 2,891; control group, preintervention 4,639 ± 2,011, postintervention 4,940 ± 2,552).

Cognitive Tests

Word fluency scores of participants in the intervention group improved significantly more than those in the control group (interaction $F(1, 128) = 6.833$, $P = .01$). There were no significant differences in other tests of delayed recall, dual task, clock drawing, abstract reasoning, TMT, DSST, or YKSST (Table 2).

Questionnaires on QOL, Mood, Functional Capacity, Range of Activity, and Social Network

The intervention group had significantly greater improvement in QOL than the control group ($F(1,128) = 9.751$, $P = .002$). A significant difference was found for functional capacity, which resulted from a significant decrease in the control group ($F(1,128) = 13.055$, $P < .001$). There was also a significant difference in all three subscales due to a significant decrease in the control group: instrumental self-maintenance ($F(1,128) = 9.801$, $P = .002$), intellectual activity ($F(1,128) = 5.543$, $P = .02$), and social role ($F(1,128) = 24.925$, $P < .001$). There were no significant differences observed in other questionnaires on mood, range of activity, or social network (Table 2).

Assessment of Motor Function

The intervention group had significantly greater improvement on the TUG than the control group ($F(1,127) = 10.117$, $P = .002$); one participant withdrew because of knee pain. There were no significant differences between the treatment and control groups in grip force, balance time, or walking speed tests, although the control group had a significant increase in grip force (Table 2).

All differences remained in the intention-to-treat analysis (word fluency score interaction $F(1, 133) = 7.420$, $P = .007$, post hoc analysis within subjects (intervention group $P = .001$, control group $P = .55$); QOL interaction $F(1, 133) = 6.936$, $P = .009$, post hoc analysis (intervention group $P = .03$, control group $P = .14$); TMIG-IC interaction $F(1, 133) = 12.035$, $P = .001$, post hoc analysis (intervention group $P = .21$, control group $P < .001$); TUG interaction $F(1, 131) = 9.013$, $P = .003$, post-hoc analysis (intervention group $P < .001$, control group $P < .001$)).

Table 1. Baseline Characteristics of Trial Participants

Characteristic	Intervention (n = 75)	Control (n = 75)	Total (N = 150)
Age, mean ± SD	71.9 ± 4.1	72.0 ± 3.9	72.0 ± 4.0
Sex, n			
Male	23	21	44
Female	52	54	106
Education, years, mean ± SD	11.8 ± 2.5	11.9 ± 2.3	11.9 ± 2.4
Mini-Mental State Examination score, mean ± SD	27.7 ± 1.9	27.9 ± 2.0	27.8 ± 1.9

SD = standard deviation.

DISCUSSION

Significant interventional benefits were shown in word fluency, QOL, functional capacity including social interaction, and motor function. Some beneficial changes were observed in the control group, such as grip force, possibly due to effects by participation.

Optimal Cognitive Health

The benefits of a walking program in a small group setting could result from synergistic effects of enhanced motivation, positive emotion, and social interaction. The importance of motivation has been emphasized in rehabilitation,[20] and cognition and emotions interact closely, based on dynamic coordination of networks in the brain.[21]

The Cognitive and Emotional Health Project proposed that optimal cognitive health is not just the absence of cognitive deficits, but also the enhancement of cognitive and emotional health to maintain social connectedness and the ability to function independently. It has also been emphasized that cognitive and emotional health should be evaluated in the context of social functioning.[12] Concerning social interaction, an interventional effect was shown in the self-reported awareness of social role (the subscale of TMIG-IC). It was reported that elderly individuals tend to feel alienation from society without a social role, and emotional isolation could be a risk factor of mental decline.[9] We assumed that the participants would feel a sense of participation in the community through intervention.

Table 2. Results of All Participants

	Mean ± Standard Deviation							
	Intervention		Control		Interaction		Post Hoc Analysis, *P*-Value	
Classification	Before	After	Before	After	*F*-Value	*P*-Value	Intervention	Control
Cognition								
Dual task test	21.2 ± 6.4	22.9 ± 6.7	19.1 ± 8.0	21.6 ± 7.1	1.176	.28	.008	<.001
Delayed recall	14.2 ± 5.2	17.3 ± 5.9	13.3 ± 5.2	16.1 ± 5.6	0.395	.53	<.001	<.001
Clock drawing	6.8 ± 0.7	6.9 ± 0.3	6.8 ± 0.7	6.9 ± 0.6	0.236	.63	.09	.31
Categorical word fluency	16.0 ± 4.0	17.2 ± 4.8	15.8 ± 4.9	15.6 ± 4.3	6.833	.01	.003	.53
Abstract reasoning	10.1 ± 3.6	10.4 ± 3.5	10.2 ± 3.5	10.8 ± 3.0	0.433	.51	.26	.04
TMT								
Part A	41.7 ± 14.8	41.2 ± 17.5	43.4 ± 15.8	43.0 ± 17.5	0.024	.88	.70	.87
Part B	119.3 ± 46.4	109.4 ± 35.0	125.9 ± 43.6	123.6 ± 49.3	1.010	.32	.06	.67
DSST	54.8 ± 12.9	58.8 ± 15.7	53.4 ± 14.4	57.4 ± 15.4	0.002	.96	<.001	<.001
YKSST	45.0 ± 11.2	48.3 ± 12.1	43.6 ± 10.5	45.7 ± 10.1	1.735	.19	<.001	.001
Questionnaire								
QOL	44.0 ± 5.8	45.3 ± 4.4	45.1 ± 5.3	44.5 ± 5.8	9.751	.002	.005	.12
GDS	3.7 ± 3.4	3.2 ± 3.0	3.4 ± 2.9	3.4 ± 3.0	2.075	.15	.04	.97
TMIG-IC	11.7 ± 1.6	11.9 ± 1.4	12.0 ± 1.4	11.6 ± 1.6	13.055	<.001	.15	<.001
LSA	94.5 ± 16.6	101.1 ± 15.4	90.4 ± 20.0	95.9 ± 18.0	0.134	.71	.002	.009
Lubben	16.1 ± 6.3	16.3 ± 5.7	17.8 ± 5.1	16.8 ± 5.2	2.033	.16	.78	.09
Motor								
Grip force	27.5 ± 6.8	28.4 ± 7.5	25.9 ± 6.9	28.1 ± 7.0	3.397	.07	.05	<.001
Balance	47.2 ± 19.2	48.6 ± 16.1	39.7 ± 21.6	40.0 ± 21.6	0.228	.63	.43	.90
TUG	5.6 ± 0.9	4.9 ± 0.7	5.7 ± 1.0	5.4 ± 0.8	10.117	.002	<.001	<.001
Speed	2.6 ± 0.4	2.5 ± 0.3	2.7 ± 0.4	2.5 ± 0.4	0.904	.34	<.001	<.001

The interaction was examined to assess the differential effect between the intervention and control groups, and post hoc analysis was conducted within-subject with Bonferroni correction.

The Trail-Making Test (TMT) was assessed according to time required, and other tests were assessed according to scores. Higher Dual task, Delayed recall, Clock drawing, Categorical word fluency, Abstract reasoning, Digit-Symbol Substitution Test (DSST), and Yamaguchi Kanji-Symbol Substitution Test (YKSST) scores and lower TMT scores indicate better performance.

Higher QOL, Tokyo Metropolitan Institute of Gerontology Index of Competence (TMIG-IC), Life Space Assessment (LSA), and Lubben Social Network Scale scores (Lubben) and lower Geriatric Depression Scale (GDS) scores indicate better performance.

Higher grip force and balance scores and lower Timed Up and Go (TUG) and speed scores indicate better performance.

Walking Program Emphasizing Mutual Support for Self-Management

This study showed that the acquisition of a walking habit is beneficial for the prevention of mental decline in elderly individuals, as previous observational studies suggested.[6,7] Exercise could have a larger effect in combination with social interaction. Animal studies suggest that greater benefits may be expected when exercise is conducted voluntarily in enriched environments (e.g., housing animals in groups in large cages with structures for exploration, physical activity, and sensorimotor learning).[22–24] The results could be applied to humans; exercise may have greater benefits when conducted voluntarily with social interactions in a pleasant atmosphere. Regarding voluntary involvement, the program gave priority to self-management. The participants would be encouraged toward a self-help effort to achieve the goal that they set for themselves, and the facilitators were required only to enhance participants' motivation in their self-managed activity. It has been recommended that rehabilitation for individuals with dementia should be based on five principles: keeping a pleasant atmosphere, enhancing participants' motivation and self-directed thinking, maintaining interactive communication, providing social roles to each participant, and errorless learning.[25] Although these principles focus on individuals with dementia, the concept underlying the present intervention was in accord with these principles. Motivation is essential for developing good habits. Interactive communication and sharing roles are helpful for smooth group activity. The role of facilitators is to keep the atmosphere pleasant and encourage group activities.

Feasibility of Implementation of the Community-Led Intervention Program

The intervention presented here is simple, and other municipalities can implement it easily and effectively. Regarding the time period, the 3-month period was determined in accordance with the Preventive Long-Term Care programs, which are implemented for a 3-month period. Previous randomized controlled trials of exercise intervention for prevention of mental decline set longer periods; one study used a 24-week home-based program of physical activity,[4] and another reported the effects of resistance training and balance training over periods of 6 months and 1 year.[26,27] The current study suggested that cognitive improvement could be achieved using a short-term 3-month intervention, although continuity is important to

ensure the positive effects, and thus a longer-term follow-up of the participants should be conducted.

Cost is a major concern because effective prevention strategies would have large public health implications in reducing economic and social burdens, especially in the face of progressive aging of the population. The walking program can be conducted at a low cost.

The walking program is simple enough that healthcare staff who have undergone effective training programs can conduct it. In this regard, municipality-led community-based rehabilitation could provide an effective application of the World Health Organization's task-shifting concept.[28] In recent years, the notion of task shifting has gained popularity as a potential means of providing care to greater numbers of patients in underresourced areas.[29] The Japanese policy of preventing mental decline takes advantage of the essence of task shifting to drive community-based rehabilitation and to organize and train volunteers as community rehabilitation facilitators. Those who have completed the intervention could become community rehabilitation facilitators to promote the prevention program throughout the community.

Limitations

One limitation of the study is that the participants might have been healthier people than the general population. The sample was self-selected, and fewer than 10% of those who received informational mailings were enrolled in the trial. Another bias was related to the difference of steps. The intervention group walked an average of 1,000 steps more at baseline than the control group.

This exploratory study lays the groundwork for a large intervention, and its efficacy should be examined with a larger population. This study could serve as the basis for implementation of a community-based intervention program to prevent mental decline.

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Tannic Acid Is a Natural β-Secretase Inhibitor That Prevents Cognitive Impairment and Mitigates Alzheimer-like Pathology in Transgenic Mice*

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Background: Recent focus has been given to anti-amyloidogenic naturally occurring polyphenols known as flavonoids.
Results: The polyphenol tannic acid prevented behavioral impairment and mitigated Alzheimer disease-like pathology.
Conclusion: Tannic acid may be prophylactic for Alzheimer disease by inhibiting β-secretase activity and mitigating brain pathology.
Significance: This nutraceutical approach offers a new class of drug for inhibiting β-secretase with few if any side effects.

Amyloid precursor protein (APP) proteolysis is essential for production of amyloid-β (Aβ) peptides that form β-amyloid plaques in brains of Alzheimer disease (AD) patients. Recent focus has been directed toward a group of naturally occurring anti-amyloidogenic polyphenols known as flavonoids. We orally administered the flavonoid tannic acid (TA) to the transgenic PSAPP mouse model of cerebral amyloidosis (bearing mutant human APP and presenilin-1 transgenes) and evaluated cognitive function and AD-like pathology. Consumption of TA for 6 months prevented transgene-associated behavioral impairment including hyperactivity, decreased object recognition, and defective spatial reference memory, but did not alter nontransgenic mouse behavior. Accordingly, brain parenchymal and cerebral vascular β-amyloid deposits and abundance of various Aβ species including oligomers were mitigated in TA-treated PSAPP mice. These effects occurred with decreased cleavage of the β-carboxyl-terminal APP fragment, lowered soluble APP-β production, reduced β-site APP cleaving enzyme 1 protein stability and activity, and attenuated neuroinflammation. As *in vitro* validation, we treated well characterized mutant human APP-overexpressing murine neuron-like cells with TA and found significantly reduced Aβ production associated with less amyloidogenic APP proteolysis. Taken together, these results raise the possibility that dietary supplementation with TA may be prophylactic for AD by inhibiting β-secretase activity and neuroinflammation and thereby mitigating AD pathology.

Alzheimer disease (AD)[3] is the most common dementia and is a growing worldwide public health concern (1). AD neuropathological hallmarks include extracellular deposits of amyloid-β (Aβ) peptides, intracellular neurofibrillary tangles, neuronal and synaptic degeneration/loss, and neuroinflammation (2). Brain Aβ deposition likely results from increased peptide accumulation/reduced clearance, endorsing toxic events that drive AD pathogenesis (3, 4). Aβ is produced from sequential endoproteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases (5–9), and enters a dynamic equilibrium between soluble and deposited forms (10). In recent years, much attention has been directed toward soluble multimeric forms of Aβ peptides as the toxic species. These so-called "Aβ oligomers" disrupt synaptic function and induce neurotoxicity *in vivo* (11–13).

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[3] The abbreviations used are: AD, Alzheimer disease; CTF, carboxyl-terminal fragment; APP, amyloid precursor protein; Aβ, amyloid-β; BACE1, β-site APP cleaving enzyme 1; CAA, cerebral amyloid angiopathy; EGCG, (−)-epigallocatechin-3-gallate; TA, tannic acid; Iba1, ionized calcium-binding adapter molecule 1; GFAP, glial fibrillary acidic protein; QPCR, quantitative real-time PCR; ANOVA, analysis of variance; CC, cingulate cortex; H, hippocampus; EC, entorhinal cortex; sAPP, soluble APP.

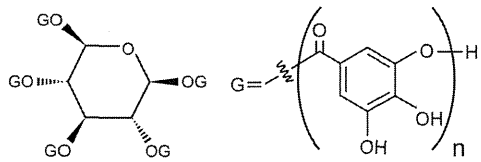

FIGURE 1. **Chemical structure of tannic acid (CAS 1401–55-4, $C_{76}H_{52}O_{46}$).** TA consists of a glucose core, which covalently connects to 3–5 gallic acid (3,4,5-trihydroxyl benzoic acid) residues through ester bonds. Each gallate residue can covalently link to other gallic acid molecules. Thus, TA is referred to as a glucoside polymer of gallic acid.

Rooted in the "amyloid cascade hypothesis" of AD, which purports that cerebral Aβ accumulation sets a toxic downstream cascade into motion (2–4), much focus has been directed toward anti-amyloid therapies. Specific approaches include reducing cerebral Aβ production or enhancing Aβ clearance (14–19). Although synthetic drugs have been anti-amyloid agents of choice, these compounds can have significant undesirable side effects, especially when given long-term in a disease prevention paradigm. For example, the ADAPT trial to test nonsteroidal anti-inflammatory drugs for primary AD prevention was prematurely halted due to nonsteroidal anti-inflammatory drug-associated cardiotoxicity (20, 21). Naturally occurring dietary compounds, or "nutraceuticals," represent an alternative class of molecules that typically have fewer side effects than designer drugs (22).

Others and we have previously reported that nutraceuticals including the green tea polyphenol (−)-epigallocatechin-3-gallate (EGCG) (23, 24), the citrus bioflavonoid luteolin (25), grape-derived polyphenols (26, 27), and caffeine (28) have anti-amyloidogenic properties. Based on our findings that EGCG enhances α-secretase APP cleavage and mitigates cerebral amyloidosis in the Tg2576 mouse model of cerebral amyloidosis (23, 24), we sought to investigate a structurally related compound, tannic acid (TA). TA is a plant-derived hydrolyzable tannin polyphenol (29) that is a gallic acid polymer glucoside ($C_{76}H_{52}O_{46}$; Fig. 1). In addition to structural similarity between TA and EGCG (both contain gallate moieties), both compounds inhibit/destabilize Aβ fibrils *in vitro* (30–32). To explore whether TA impacted AD-like features, we orally administered the compound for 6 months to the doubly transgenic (APP + $PS1_{\Delta E9}$) PSAPP mouse model of cerebral amyloidosis and examined behavioral impairment, AD-like pathology, APP processing, and neuroinflammation. Additionally, we validated our results *in vitro* using mutant human APP-overexpressing murine neuron-like cells.

EXPERIMENTAL PROCEDURES

Mice—Male double transgenic "Swedish" $APP_{K670N/M671L}$ (APPswe) plus Presenilin 1 exon 9 deleted ($PS1_{\Delta E9}$) B6C3-Tg 85Dbo/J mice on a C57BL/6xC3H background (designated PSAPP mice) were obtained from the Jackson Laboratory (Bar Harbor, ME) and were bred with female C57BL/6 mice to yield mutant PSAPP (APPswe + $PS1_{\Delta E9}$) and wild-type (WT) offspring. PSAPP mice overproduce human $A\beta_{1-40}$ and $A\beta_{1-42}$ peptides and develop progressive cerebral β-amyloid deposits and learning and memory impairment (33–36). All mice were characterized by PCR genotyping for mutant human APP and PS1 transgenes as described elsewhere (35). We strictly used PSAPP and WT littermates obtained from this breeding strategy for all analyses. Thus, all mice used in this study are genetically comparable.

TA was obtained from Sigma, resuspended in distilled water, and orally administered to 16 PSAPP mice (PSAPP-TA mice; 8 males and 8 females). As a vehicle control, 16 additional PSAPP mice received distilled water (PSAPP-V mice; 8 males and 8 females). In addition, 32 WT littermates received TA (WT-TA mice; 8 males and 8 females) or distilled water (WT-V mice, 8 males and 8 females). Beginning at 6 months of age, animals were gavaged with TA (30 mg/kg) or vehicle once daily for 6 months. In parallel, to examine if PSAPP mice were cognitively impaired at the initiation of dosing and whether TA treatment prevented *versus* delayed kinetics of disease progression, 12 untreated PSAPP mice (PSAPP-6M, 6 males and 6 females) and 12 untreated WT mice (WT-6M, 6 males and 6 females) at 6 months of age were included for analyses of behavior, β-amyloid pathology, and neuroinflammation. Mice were housed in a specific pathogen-free barrier facility under a 12/12-h light-dark cycle, with *ad libitum* access to food and water. All experiments were performed in accordance with the guidelines of the Animal Use Ethics Committee of the Saitama Medical University and of the NIH.

Behavioral Analyses—Two weeks prior to sacrifice, a battery of behavioral tests was conducted to assess exploratory activity, novel-object recognition and memory retention, and spatial learning and memory in the six groups of mice detailed above. Exploratory activity was evaluated by individually placing mice into a novel environment (the left corner of a white polyethylene chamber; 54 × 39 × 20 cm). Their activity was recorded for 20 min by an overhead video camera (BL-C131, Panasonic, Fukuoka, Japan) connected to a Windows PC, and horizontal locomotion and rearing scores were counted for each 2-min time bin (37, 38). The next day, novel-object recognition and memory retention were assessed as described (39). Briefly, each mouse was habituated in a cage for 4 h, and then two different shaped objects were concurrently provided to the mouse for 10 min. The number of times that the mouse explored the object (defined as number of instances where a mouse directed its nose 2 cm or less distance from the object) that was later replaced by a novel object was counted for the initial 5 min of exposure (training phase). To test memory retention on the following day, one of the original objects was replaced with a different shaped novel object, and then the number of explorations of the novel object was counted for 5 min (retention test). The recognition index, taken as an index of memory, is reported as frequency (%) of explorations of the novel *versus* original objects.

Subsequently, Morris water maze testing was performed essentially as previously described (40, 41). The water maze consisted of a circular pool (80 cm diameter) filled with water maintained at 23–26 °C. For the purpose of post hoc analyses, the pool was divided into quadrants (Q1 to Q4), and a 6-cm diameter plexiglass platform was located 1 cm above the water surface in the center of Q2. After a minimum of 20 min habituation to the room, mice naïve to the test were placed in the pool and allowed to search for the platform for 60 s. On the first 2 days (four trials were conducted per day with a 20-min inter-

trial interval), a visible cue was placed on the platform and its location was randomly varied among four possible locations (counterbalanced across mice). The trial ended when a mouse climbed the platform, or in the allocated 60 s, whichever came first. After finding and climbing on the platform, each mouse was allowed to remain there for 20 s, and was then returned to its cage. Animals that did not locate the platform within 60 s were guided to it and allowed to remain there for 20 s before being returned to their cages. On the third day, submerged platform testing was conducted for five consecutive days (learning phase; four trials per day with a 20-min inter-trial interval). The location of the indiscernible platform remained in Q2, 1 cm below the water surface, and mice were placed into the pool in one of seven randomly selected locations (excluding the position immediately adjacent to the platform). One day after the conclusion of the learning phase, memory retention was determined in a single 60-s probe trial. The submerged platform was removed from the water maze, and mice were placed and released opposite the site where the platform had been located and time spent in each quadrant was recorded for the probe trial. All behavioral tests were performed in a room (6 m × 4.5 m) with indirect lighting and multiple visible cues on the walls. The examiner determined the time of swimming until the mouse reached the platform (latency) using a stopwatch. In addition, trials were recorded using an overhead video camera and were analyzed using customized macro software in Microsoft Excel. All trials were performed at the same time of day (±1 h), during the animals' light phase. So as not to interfere with behavioral testing, TA or vehicle treatment was carried out 1 h after behavioral testing.

Tissue Preparation—Tissue was processed according to our previously described methods (16, 18, 42, 43). At 12 months of age, animals were anesthetized with sodium pentobarbital (50 mg/kg) and euthanized by transcardial perfusion with ice-cold physiological saline containing heparin (10 units/ml). Brains were isolated and quartered (sagittally at the level of the longitudinal fissure of the cerebrum, and then coronally at the level of the anterior commissure) using a mouse brain slicer (Muromachi Kikai, Tokyo, Japan). Right anterior cerebral quarters were weighed and snap-frozen at −80 °C for α- or β-secretase activity analyses. Right posterior cerebral quarters were further divided into two pieces, and weighed and snap-frozen at −80 °C. One-half was sequentially extracted in Tris-buffered saline (TBS; 25 mM Tris-HCl, pH 7.4, 150 mM NaCl), 2% SDS-, and guanidine-soluble fractions for Aβ sandwich enzyme-linked immunosorbent assays (ELISAs). The other half was used for holo-APP, β-site APP cleaving enzyme 1 (BACE1), and β-carboxyl-terminal fragment (β-CTF: phospho-C99 and C99) Western blots. Left anterior cerebral quarters were weighed and immersed in RNA stabilization solution (RNAlater®, Applied Biosystems, Foster City, CA) and then snap-frozen at −80 °C for proinflammatory cytokine and BACE1 quantitative real-time PCR (QPCR) analyses. Left posterior cerebral quarters were immersion fixed in 4% paraformaldehyde in 0.1 M phosphate buffer at 4 °C overnight, and routinely processed in paraffin for immunohistochemical analyses.

Immunohistochemistry—For paraffin blocks, we sectioned five coronal sections (per set) with a 100 μm interval and a thickness of 5 μm for each brain region (for cingulate cortex (CC), bregma −0.10 to −0.82 mm; for hippocampus (H) and entorhinal cortex (EC), bregma −2.92 to −3.64 mm) (44). We prepared three sets of five sections in each separate region for analyses of Aβ deposits/β-amyloid plaques (for burden, plaque number, and maximum diameter morphometry) as well as ionized calcium-binding adapter molecule 1 (Iba1, to mark reactive microglia) and glial fibrillary acidic protein (GFAP, an astrocytosis marker) burdens. Immunohistochemical staining was conducted according to the manufacturer's protocol using a Vectastain ABC *Elite* kit (Vector Laboratories, Burlingame, CA) coupled with the diaminobenzidine reaction, except that the biotinylated secondary antibody step was omitted for Aβ immunohistochemical staining. The following primary antibodies were used: a biotinylated human Aβ monoclonal antibody (4G8; 1:200, Covance Research Products, Emeryville, CA), Iba1 polyclonal antibody (1:1,000, Wako, Osaka, Japan), and GFAP polyclonal antibody (1:500, Dako, Carpinteria, CA). Using additional sets of five sections, normal mouse or rabbit serum (isotype control) or phosphate-buffered saline (0.1 M PBS, pH 7.4) was used instead of primary or secondary antibody or ABC reagent as negative controls.

Image Analysis—Quantitative image analysis was done based on previously validated methods (16, 18, 42, 43). Images were acquired as digitized tagged-image format files to retain maximum resolution using a BX60 microscope with an attached CCD camera system (DP-70, Olympus, Tokyo, Japan), and digital images were routed into a Windows PC for quantitative analyses using SimplePCI software (Hamamatsu Photonics, Hamamatsu, Shizuoka, Japan). We captured images of five 5-μm sections through each anatomic region of interest (CC, EC, and H) based on anatomical criteria defined by Franklin and Paxinos (44), and obtained a threshold optical density that discriminated staining from background. Each anatomic region of interest was manually edited to eliminate artifacts. For Aβ, Iba1 (microgliosis), and GFAP (astrocytosis) burden analyses, data are reported as the percentage of labeled area captured (positive pixels) divided by the full area captured (total pixels). Selection bias was controlled for by analyzing each region of interest in its entirety.

For β-amyloid plaque morphometric analyses, diameters (based on maximum length) of β-amyloid plaques were measured, and numbers of β-amyloid plaques falling into three mutually exclusive diameter categories (<25, 25–50, or >50 μm) were tabulated. Results are presented as mean plaque number per mouse in each region examined. For cerebral amyloid angiopathy (CAA) morphometric analysis, we counted numbers of Aβ antibody-stained cerebral vessels in each anatomic region of interest based on our previous methods (43); those data are shown as mean CAA deposit number per mouse.

Cell Culture—The N2a cell line that stably overexpresses human "Swedish"-mutated APP-695 (SweAPP N2a cells) was kindly provided by Dr. Gopal Thinakaran (Department of Neurobiology, University of Chicago). SweAPP N2a cells were grown in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, 2 mM glutamine, 100 units/ml of penicillin, 0.1 μg/ml of streptomycin, and 200 μg/ml of G418 sulfate according to previously described methods (16, 23, 25).

SweAPP N2a cells were seeded in 24-well tissue culture plates at 1×10^5 cells per well. Cultured cells were differentiated into neuron-like cells by 2 h pre-treatment with neurobasal media containing 300 μM dibutyryl cAMP and then treated with TA (1.563, 3.125, 6.25, 12.5, or 25 μM) or 0.1 M PBS (pH 7.4; as vehicle control) for 12 h in the same media prior to analyses.

Lactate Dehydrogenase Release Assay—SweAPP N2a cells were seeded in 24-well tissue culture plates at 1×10^5 cells per well. Culture cells were differentiated into neuron-like cells by 2 h pre-treatment with neurobasal media containing 300 μM dibutyryl cAMP and then treated with TA (3.125, 6.25, 12.5, or 25 μM) or 0.1 M PBS (pH 7.4; vehicle control) for 12 h in the same media. Culture wells were then assayed for cell death by a lactate dehydrogenase release assay (Promega) as described (45).

Cell-free BACE1 Activity Assay—To directly test the effect of TA on BACE1 activity, we used available kits based on secretase-specific peptides conjugated to DABCYL/EDANS fluorogenic reporter molecules (Cayman Chemical, Ann Arbor, MI) in accordance with the manufacturer's instructions. Briefly, BACE1 enzyme was incubated with various concentrations of TA (1.563, 3.125, 6.25, 12.5, or 25 μM) or BACE1 inhibitor II (1.25 μm; as a positive control) in the presence of 1× reaction buffer for 40 min prior to reading fluorescence values on a FLUOstar Omega (BMG LABTECH, San Diego, CA) fluorescent microplate reader.

ELISA—We separately quantified $A\beta_{1-40}$ and $A\beta_{1-42}$ in brain homogenates and cultured SweAPP N2a cell supernatants by sandwich ELISAs. Brain $A\beta_{1-40}$ and $A\beta_{1-42}$ species were detected by a three-step extraction protocol according to previously published methods (46, 47). Briefly, we homogenized brains using TissueLyser LT (Qiagen, Valencia, CA; two times for 1 min at 50 Hz) in TBS solution containing protease inhibitor mixture (Sigma), centrifuged homogenates at 18,800 × *g* for 60 min at 4 °C, and removed the supernatants (TBS-soluble fraction). Resulting pellets were treated with 2% SDS in H_2O with the same protease inhibitors and homogenized using TissueLyser LT (one time for 1 min at 50 Hz). We then centrifuged the homogenates at 18,800 × *g* for 60 min at 4 °C and collected supernatants (2% SDS-soluble fraction). Finally, the remaining pellets were treated with 5 M guanidine HCl and dissolved by occasional mixing on ice for 30 min, then centrifuged at 18,800 × *g* for 60 min at 4 °C, and supernatants were collected representing the guanidine HCl-soluble fraction.

$A\beta_{1-40}$ and $A\beta_{1-42}$ species were separately quantified in individual samples in duplicate using ELISA kits (catalogue number 27718 for $A\beta_{1-40}$ and number 27712 for $A\beta_{1-42}$; IBL, Fujioka, Gunma, Japan) in accordance with the manufacturer's instructions (48). We also quantified Aβ oligomers in the 2% SDS-soluble fraction in duplicate individual samples by Aβ oligomer ELISA (catalogue number 27725; IBL) according to the manufacturer's instructions (49). All samples fell within the linear range of the standard curve. $A\beta_{1-40}$ and $A\beta_{1-42}$ ELISA values are reported as picograms of $A\beta_{1-x}$/wet mg of brain, and the Aβ oligomer concentration is reported as picomolar.

Western Blot—Cultured SweAPP N2a cells were treated with various doses of TA (1.563, 3.125, 6.25, 12.5, or 25 μM) or 0.1 M PBS (pH 7.4; as a vehicle control) for 12 h. Cultured cells were then lysed in ice-cold lysis buffer (containing 20 mM Tris-HCl, pH 7.5, 150 mM NaCl, 1 mM Na_2EDTA, 1 mM EGTA, 1% Triton X-100, 2.5 mM sodium pyrophosphate, 1 mM β-glycerophosphate, 1 mM Na_3VO_4, 1 μg/ml of leupeptin, and 1 mM PMSF). Mouse brain homogenates were lysed in TBS solution containing protease inhibitor mixture (Sigma) followed by TNE buffer (10 mM Tris-HCl, 1% NP-40, 1 mM EDTA, and 150 mM NaCl), and aliquots corresponding to 10 μg of total protein were electrophoretically separated using 10 or 15% Tris glycine gels based on target protein molecular weights. Electrophoresed proteins were transferred to polyvinylidene difluoride membranes (Bio-Rad) that were subsequently blocked in blocking buffer (1% (w/v) nonfat dry milk in TBS containing 0.1% (v/v) Tween 20) for 1 h at ambient temperature. After blocking, membranes were hybridized for 1 h at ambient temperature with primary antibodies: amino-terminal APP polyclonal antibody (1:400, IBL), carboxyl-terminal soluble APP-α (sAPP-α) monoclonal antibody (2B3; 1:100, IBL) directed against amino acids DAEFRHDSGYEVHHQK, carboxyl-terminal soluble APP-β (sAPP-β) monoclonal antibody that recognizes Swedish mutant (ISEVNL) protein (6A1; 1:100, IBL), carboxyl-terminal BACE1 polyclonal antibody (1:400, IBL), amino-terminal Aβ monoclonal antibody (82E1; 1:150, IBL), carboxyl-terminal APP polyclonal antibody (1:1,000, Merck Millipore, Billerica, MA), or actin polyclonal antibody as a loading control (1:500, Santa Cruz Biotechnology, Santa Cruz, CA). Membranes were then rinsed three times for 30 min each in TBS containing 0.1% (v/v) Tween 20 and incubated for 1 h at ambient temperature with appropriate horseradish peroxidase-conjugated secondary antibodies. After additional rinsing as above, membranes were incubated for 5 min at ambient temperature with enhanced chemiluminescence substrate (SuperSignal West Dura Extended Duration Substrate, Thermo Fisher Scientific, Waltham, MA), exposed to film, and developed.

Secretase Activity Assays—For α- and β-secretase activity analyses in brain homogenates, we used available kits based on secretase-specific peptides conjugated to fluorogenic reporter molecules (DABCYL/EDANS; R & D Systems, Minneapolis, MN) according to our published methods (23, 43). Briefly, brains were lysed in ice-cold 1× cell extraction buffer for 10 min and centrifuged at 18,800 × *g* for 1 min. Supernatants were collected and kept on ice. Appropriate amounts of brain homogenate, reaction buffer, and fluorogenic substrate were added in duplicate to a 96-well plate and incubated in the dark at 37 °C for various periods of time. Following incubation, fluorescence was monitored (335 nm excitation and 495 nm emission) at 25 °C using a SH-9000 microplate fluorimeter with SF6 software (CORONA ELECTRIC, Hitachinaka, Ibaraki, Japan). Background was determined from negative controls (omission of brain homogenate or fluorogenic substrate).

QPCR—We quantified tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), BACE1, and β-actin mRNA levels by QPCR. Total RNA was extracted using the RNeasy Mini Kit (Qiagen), and first strand cDNA synthesis was carried out using the QuantiTect Reverse Transcription Kit (Qiagen) in accordance with the manufacturer's instructions. We diluted cDNA 1:1 in H_2O and carried out QPCR for all genes of interest using

cDNA-specific TaqMan primer/probe sets (TaqMan Gene Expression Assays, Applied Biosystems) on an ABI 7500 Fast Real-time PCR instrument (Applied Biosystems). Each 20-μl reaction mixture contained 2 μl of cDNA with 1 μl of TaqMan Gene Expression Assay reagent, 10 μl of TaqMan Fast Universal PCR Master Mix (Applied Biosystems), and 7 μl of H_2O. Thermocycler conditions consisted of: 95 °C for 15 s, followed by 40 cycles of 95 °C for 1 s and 60 °C for 20 s. TaqMan probe/primer sets were as follows: mouse TNF-α (catalogue number Mm00443258_m1), mouse IL-1β (number Mm00434228_m1), mouse BACE1 (number Mm00478664_m1), and mouse β-actin (number Mm00607939_s1; used as an internal reference control) (Applied Biosystems). Samples that were not subjected to reverse transcription were run in parallel as negative controls to rule out genomic DNA contamination (data not shown). A "no template control" was also included for each primer set (data not shown). The cycle threshold number (C_T) method (50) was used to determine relative amounts of initial target cDNA in each sample. Results for BACE1 expression are expressed relative to vehicle-treated WT mice, whereas TNF-α and IL-1β expression values are normalized to WT-6M littermates.

Statistical Analysis—All experiments were performed by an examiner blinded to sample identities, and code was not broken until the analyses were completed. Data are presented as the mean ± 1 S.E. A hierarchical analysis strategy was used for time-dependent behavioral data in which the first step was a repeated-measures analysis of variance (ANOVA) to assess the significance of the main effects and interactive terms. If significant, post hoc testing was done with Tukey's HSD or Dunnett's T3 methods, and appropriate p values are reported based on adjustment according to Levene's test for equality of the variance. For all other data, in instances of single mean comparisons, Levene's test followed by t test for independent samples was performed. In instances of multiple mean comparisons, one-way ANOVA was used, followed by post hoc comparison of the means using Bonferroni's or Dunnett's T3 methods (where appropriateness was determined using Levene's test). A p value of less than 0.05 was considered to be significant. All analyses were performed using the Statistical Package for the Social Sciences, release IBM SPSS 19.0 (IBM, Armonk, NY).

RESULTS

Oral Tannic Acid Treatment Mitigates Hyperactivity and Cognitive Impairment in PSAPP Mice—We began by orally administering TA or vehicle to PSAPP or WT mice starting at 6 months of age for a period of 6 months and subsequently conducted a behavioral testing battery. In addition, to examine cognitive status when dosing started, untreated PSAPP and WT mice at 6 months of age were included for analyses of behavior. When placed into a novel environment, PSAPP-V mice were hyperactive as measured by higher locomotion and rearing scores compared with the other 5 groups of mice (Fig. 2*A*). This behavioral phenotype has been observed in mouse models of cerebral amyloidosis (*e.g.* Tg2576 or PSAPP) (18, 34, 51), and may be associated with disinhibition resulting from cortical and/or hippocampal injury (38). Overall ANOVA showed main effects of time ($p < 0.001$), genotype ($p < 0.001$), and treatment ($p < 0.001$), and post hoc comparisons showed statistically significant differences between PSAPP-V mice and the other 5 mouse groups at each time point for locomotion scores (Fig. 2*A*, *, $p < 0.05$ for PSAPP-V *versus* PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice) and for rearing scores (Fig. 2*A*, *, $p < 0.05$ for PSAPP-V *versus* PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). Hyperactivity was fully prevented in PSAPP-TA mice, as they did not statistically differ from WT-V WT-TA, PSAPP-6M, or WT-6M mice ($p > 0.05$).

We then tested learning and memory in the same cohort of mice by a novel object recognition assay. If mice remember an initial encounter with a novel object, they tend to preferentially explore the new *versus* familiar object, typically operationalized as "recognition index" (39). Although all groups performed similarly during the training phase of the test, in the retention phase, one-way ANOVA followed by post hoc comparison showed statistically significant differences on recognition index between PSAPP-V mice and the other 5 mouse groups as indicated (Fig. 2*B*, *, $p < 0.05$ for PSAPP-V *versus* PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). Importantly, PSAPP-TA mice had significantly increased novel object exploration frequency *versus* PSAPP-V animals (Fig. 2*B*), but did not significantly differ from WT-V, WT-TA, PSAPP-6M, or WT-6M groups ($p > 0.05$), showing that TA also prevented novel object recognition impairment associated with PSAPP transgene expression.

We further tested animals in the Morris water maze, a widely accepted assay of spatial reference learning and memory in rodents (40, 41). For the learning phase of the test, overall ANOVA showed main effects of time ($p < 0.001$) and genotype ($p < 0.001$), and post hoc comparison revealed statistically significant differences between PSAPP-V mice and the other 5 mouse groups as indicated (Fig. 2*C*, *, $p < 0.05$ for PSAPP-V *versus* PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). PSAPP-V mice had greater latency to reach the platform location after training than the other 5 mouse groups, whereas PSAPP-TA mice showed significant improvement, indicating that oral TA treatment inhibited PSAPP transgene-associated impaired spatial reference learning. For the probe trial (day 6 of testing), the invisible platform was removed from the pool and platform location memory was evaluated. When considering Q2 (goal quadrant) data, one-way ANOVA and post hoc testing showed statistically significant differences between PSAPP-V mice and the other 5 mouse groups as indicated (Fig. 2*D*, *, $p <$ 0.05 for PSAPP-V *versus* PSAPP-TA, WT-V, WT-TA, PSAPP-6M, or WT-6M mice). PSAPP-TA mice swam in the goal quadrant significant longer than PSAPP-V mice, and their behavior did not significantly differ from WT-V, WT-TA, PSAPP-6M, or WT-6M mice, showing that TA treatment prevents PSAPP transgene-associated spatial memory impairment.

It is unlikely that behavioral differences in the Morris water maze were due to motivational issues or to locomotor impairment, as there were no significant between group differences ($p > 0.05$) on swim speed during either the learning or probe trial phases of the test. Moreover, it is important to note that the degree of thigmotaxis could indicate levels of anxiety and impact interpretation of Morris water maze results. In this regard, we did not observe evidence of thigmotaxis, operationalized as prolonged movement of the mice along the pool cir-

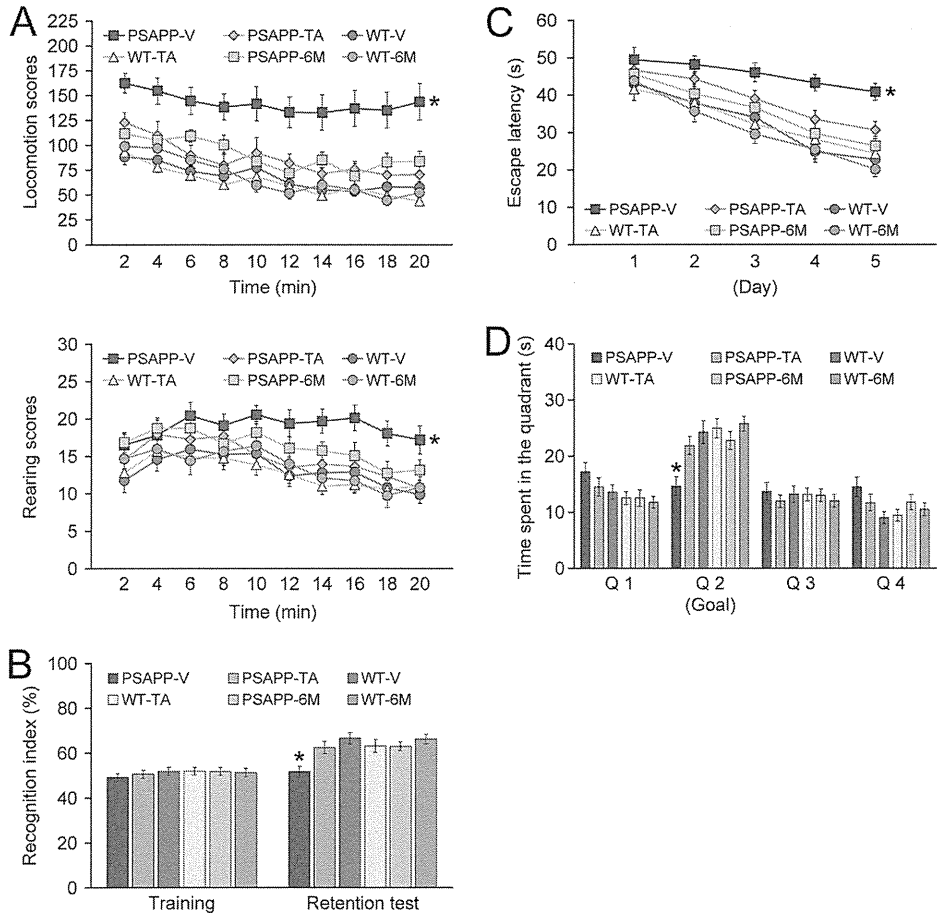

FIGURE 2. **Tannic acid treatment prevents behavioral impairment in PSAPP mice.** PSAPP mice received oral vehicle (*PSAPP-V*, $n = 16$) or TA (*PSAPP-TA*, $n = 16$) treatment, and wild-type mice were given vehicle (*WT-V*, $n = 16$) or TA (*WT-TA*, $n = 16$) orally for 6 months beginning at 6 months of age, and subjected to behavioral testing at 12 months of age. To examine cognitive status when dosing started, untreated 6-month-old PSAPP mice (*PSAPP-6M*, $n = 12$) and WT mice (*WT-6M*, $n = 12$) were included in the behavioral analyses. *A*, locomotion and rearing scores obtained from open field activity testing are shown. *B*, recognition index (%) in the object recognition test is shown (*left*, training phase; *right*, retention test phase). *C*, Morris water maze test data are shown from the submerged platform (learning phase) and from *D*, a single 60-s probe trial test (conducted 1 day after termination of the learning phase). All statistical comparisons are *versus* PSAPP-V mice.

cumference, in any mice examined during either the learning or probe trial phases of the test. Furthermore, untreated PSAPP mice at 6 months of age (PSAPP-6M) did not clearly show cognitive impairment as compared with WT mice at the same age or to WT-V or WT-TA mice at 12 months of age by open field, object recognition, or either the learning or probe trial phases of Morris water maze testing. Importantly, behavioral testing performance in PSAPP-TA mice was not significantly different from PSAPP-6M animals in each of the tests conducted. This result can be interpreted as prevention of cognitive impairment in PSAPP mice by a 6-month treatment regimen of TA. Finally, for all of the behavioral tests conducted, we used multiple ANOVA models with gender as a categorical covariate, but did not detect significant gender main effects or interactive terms ($p > 0.05$). We also stratified by gender and found a similar pattern of results as above in both males and females (data not shown).

Tannic Acid Treatment Ameliorates Aβ Pathology in an Accelerated Mouse Model of Cerebral Amyloidosis—We next evaluated Aβ/β-amyloid pathology by three strategies: Aβ antibody immunoreactivity (conventional β-amyloid "burden" analysis), β-amyloid morphometric analysis, and separate $A\beta_{1-40}$ and $A\beta_{1-42}$ sandwich ELISAs. PSAPP-V mice showed typical β-amyloid deposition (33, 36), which was significantly reduced by 51–58% in CC, EC, and H regions of PSAPP-TA mouse brains (Fig. 3, *A* and *B*, ***, $p < 0.001$). Of note, PSAPP-TA plaques were not completely attenuated *versus* PSAPP-6M mice (Fig. 3, *A* and *B*), indicating that TA mitigated as opposed to completely prevented cerebral amyloid deposition. TA reduction in β-amyloid deposits was independent of gender, being evident in both male and female PSAPP-TA *versus* PSAPP-V mice (data not shown). To assess whether reduced β-amyloid burden was specific to a particular plaque size subset or occurred more generally, we performed morphometric analysis of β-amyloid plaques in PSAPP-V and PSAPP-TA mice. According to previously described methods (18, 16, 42, 43), plaques were assigned to one of three mutually exclusive categories according to maximum diameter: small (< 25 μm), medium (between 25 and 50 μm), or large (>50 μm). Data showed that all three subsets of plaques were significantly reduced in PSAPP-TA *versus* PSAPP-V mice across all three brain regions examined (Fig. 3, *A* and *C*, ***, $p < 0.001$, % reduc-

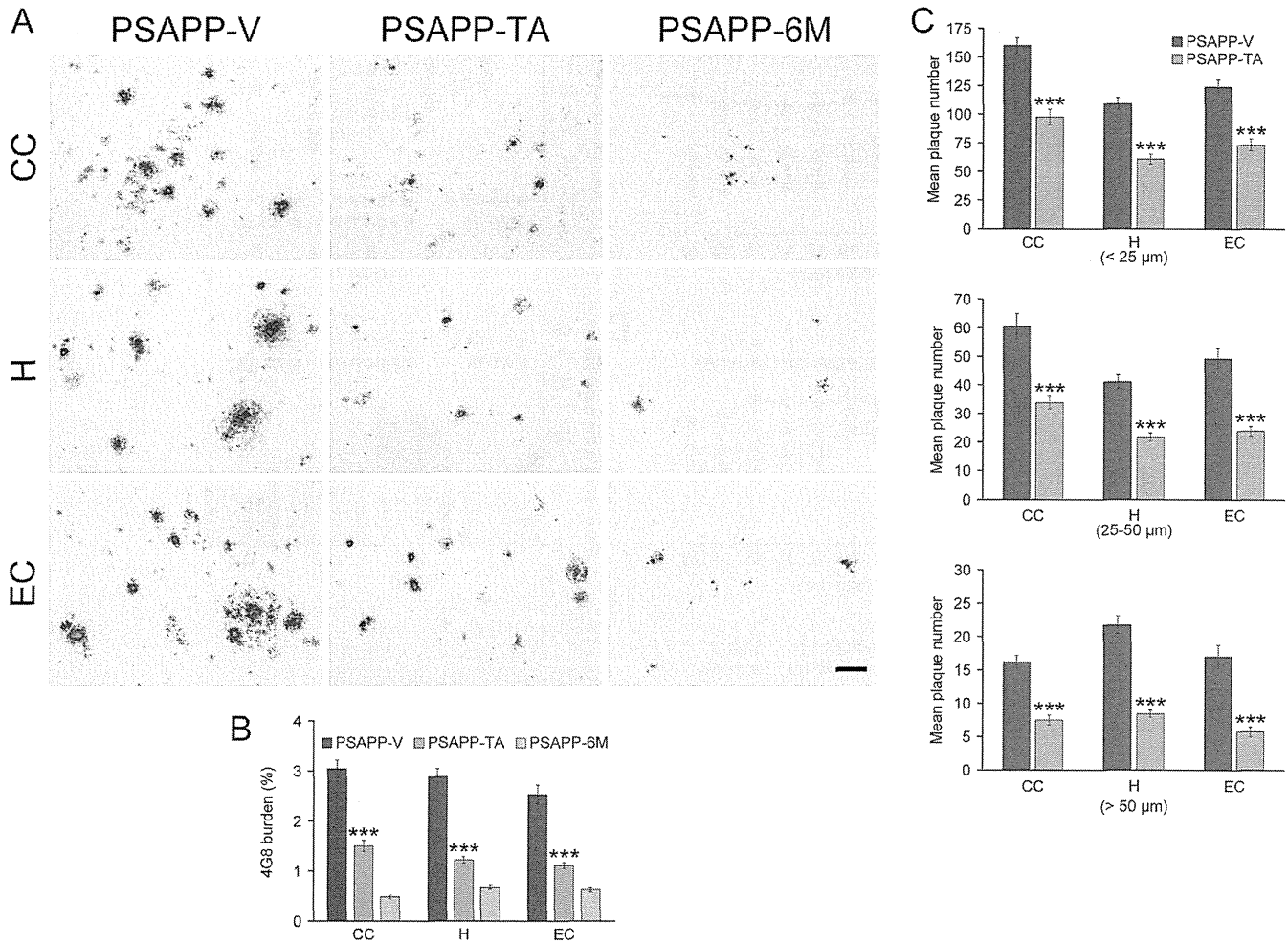

FIGURE 3. **Cerebral parenchymal β-amyloid deposits are mitigated in tannic acid-treated PSAPP mice.** Data were obtained from PSAPP mice treated with vehicle (*PSAPP-V*, $n = 16$) or TA (*PSAPP-TA*, $n = 16$) for 6 months commencing at 6 months of age (mouse age = 12 months at sacrifice). To examine if the TA treatment delayed *versus* prevented disease progression, untreated PSAPP mice (*PSAPP-6M*, $n = 12$) at 6 months of age were also included in these analyses. *A*, representative photomicrographs of 4G8 immunohistochemistry are shown for cerebral β-amyloid plaques in PSAPP-V, PSAPP-TA, and PSAPP-6M mice. Brain regions shown include: cingulate cortex (*CC*, *top*), hippocampus (*H*, *middle*), and entorhinal cortex (*EC*, *bottom*). *Scale bar* denotes 50 μm. *B*, quantitative image analysis for Aβ (4G8) burden is shown, and each brain region is indicated on the *x* axis. *C*, morphometric analysis of cerebral parencymal β-amyloid plaques is shown in PSAPP-V and PSAPP-TA mice. Brain sections were stained with 4G8 antibody, and plaques were counted based on maximum diameter and assigned to one of three mutually exclusive categories: small (<25 μm; *top*), medium (between 25 and 50 μm; *middle*), or large (>50 μm; *bottom*). Mean plaque subset number per mouse is shown on the *y* axis, and each brain region is represented on the *x* axis. The statistical comparison for *B* is within the brain region and *versus* PSAPP-TA mice, and for *C*, between PSAPP-V and PSAPP-TA mice.

tion for: small, 39–44%; medium, 44–52%; large, 54–66% plaque subsets). We stratified by gender and observed the same pattern of results in both males and females (data not shown). Untreated PSAPP-6M mice had quantitatively minor (0.5–0.7%) cerebral β-amyloid burden (Fig. 3, *A* and *B*), and the majority of these deposits were seed-like dots <25 μm in size, with only a few plaques between 25 and 50 μm. Because of this, we were unable to perform morphometric analysis of Aβ deposits for the PSAPP-6M group.

In addition to brain parenchymal deposition of β-amyloid as senile plaques, 83% of AD patients present with β-amyloid deposits in cerebral vessels, known as CAA (52). PSAPP mice also develop vascular β-amyloid deposits with age (36), and we found almost no evidence for CAA in young PSAPP-6M mice (data not shown). Having shown that TA treatment led to reduced brain parenchymal β-amyloid plaques in PSAPP mice, we wondered if cerebral vascular β-amyloid deposits might also be altered. In both PSAPP mouse groups, cerebral vascular β-amyloid deposits were predominantly detected within penetrating arteries at the pial surface in CC and EC regions and within small arteries at the hippocampal fissure. We scored Aβ antibody (4G8)-stained cerebral vascular deposits in PSAPP-V and PSAPP-TA mice and found reductions in PSAPP-TA mouse brains that reached statistical significance in all three brain regions examined (Fig. 4, *A* and *B*, **, $p < 0.01$).

In agreement with histological observations, biochemical analysis of Aβ species in brain homogenates revealed significant reductions in TBS-soluble $A\beta_{1-40}$ and in both $A\beta_{1-40}$ and $A\beta_{1-42}$ abundance in the detergent-soluble fraction from PSAPP-TA *versus* PSAPP-V mice (Fig. 4*C*, 25–33% depending on the particular Aβ species; *, $p < 0.05$). Similarly, extraction of the insoluble pellet in guanidine HCl revealed significant reductions in PSAPP-TA mice for both $A\beta_{1-40}$ and $A\beta_{1-42}$ levels (27–28%) by biochemical analysis (Fig. 4*C*, ***, $p < 0.001$).