Article

Effects of Pet Insects on Cognitive Function Among the Elderly: An fMRI Study

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Abstract: (1) Background: Animal-assisted therapy has positive effects on cognitive function, depression, performance ability, and social functioning in elderly patients. The aim of this study was to evaluate the effects of rearing pet insects on the cognitive function of healthy elderly participants, with fMRI (functional magnetic resonance imaging) being used for this purpose. (2) Methods**:** Community-dwelling elderly women (≥60 years) with normal cognitive function were enrolled during April 2015. They were randomized at a 1:1 ratio into two groups: insect-rearing and control (n=16) groups, with the insect-rearing group being further classified into two groups for analysis according to the subjects' scores in the Wisconsin Card Sorting Test, WCST) at the first fMRI: insect-rearing group I with a relatively high score (n=13), and insect-rearing group II with a relatively low score (n=6). All subjects were educated on a healthy lifestyle for better cognitive function at every visit, and the insect-rearing groups received and reared crickets as pet insects. The fMRI was performed at baseline and after 8 weeks using the WCST as a stimulus. The WCST consisted of two variations, a high level baseline (HLB) and semi-WCST version. (3) Results: There were no significant differences in the baseline characteristics among the three groups. There was a significant difference accuracy of the HLB–semi-WCST (p<0.05) in insect-rearing group II after 8 weeks from the baseline test. In the fMRI analysis involving the WCST reaction test, increased activation was observed in the right dorsal lateral prefrontal cortex and parietal cortex in insect-rearing group II when the semi-WCST, rather than the HLB, was performed. There were no significant differences in the other groups. (4) Conclusion: The rearing of pet insects as an animal-assisted therapy is cost-effective, easy, and occupies little space. In this study, it showed positive effects on executive functions and performance improvement in elderly women. Further larger studies on the effects of pet insects on cognitive function are warranted.

Keywords: cognitive function; pet insects; animal-assisted therapy; Wisconsin Card Sorting Task; functional magnetic resonance imaging; elderly women

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1. Introduction

People aged 65 or older make up 14.2% of the South Korean population, and South Korea is one of the countries where the population is aging rapidly [1]. Elderly patients have a high prevalence of comorbidity, and many of them take multiple medications, that is, polypharmacy is common, with an increased risk of medication-related complications, which results in a subsequent poor quality of life. Furthermore, medical expenses for the elderly constitute a third of the total medical expenses in South Korea, which results in a burden not only for individuals but also for the nation [1]. There is therefore a need to find noninvasive and economical treatment tools for the elderly, and physicians should play an important role in finding these tools.

Various studies have tried to use animal-assisted therapy (AAT) to treat physical and mental illnesses, and it has shown some positive results, especially in the elderly. These positive results include improvements in cognitive function, depression, performance ability, and social function [2-4]. AAT also showed beneficial results in elderly people with psychiatric disorders (dementia, depression, and schizophrenia), as well as in relatively healthy elderly subjects [2-7]. AAT is a noninvasive and relatively safe intervention that has been shown to have good potential as a useful alternative therapy for neuropsychotherapy. A diverse range of species have been used for AAT; however, most studies have involved mammals such as dogs and cats [2]. Insects could be used as a species of mediators, and pet insect-associated therapy is a new area. The area related insects have recently been expanded to not only agriculture, but also other areas, because the demand for the culture insect industry is growing. For example, the educational curriculum for children(elementary school students) has included natural organisms such as insects for science classes and activities [8].

The rearing of insects costs less than that of animals and occupies less space, and they are relatively easy to care for. It is therefore expected that they can be used for therapeutic interventions in the elderly. A previous study reported the possibility of the beneficial effects of pet insects on cognitive function and depression scores in the community-dwelling elderly [9]. However, this study had a limitation in that it depended on questionnaires to verify cognitive function. Functional magnetic resonance imaging (fMRI) measures brain activation using changes in deoxyhemoglobin in specific regions in reaction to stimuli [10, 11]. In particular, evaluation of visuospatial working memory tasks is an effective tool for assessing cognitive performance [12, 13]. To the best of our knowledge, there is no study using fMRI to assess the effect of AAT on cognitive function. Therefore, this study aimed to use fMRI to evaluate the effects of rearing pet insects on the cognitive functioning of elderly subjects.

2. Methods

2.1. Subject Eligibility

Community-dwelling right-handed elderly women (≥60 years) with normal cognitive function (Mini-Mental State Examination [MMSE] score ≥24) were enrolled during April 2015 through a community center in South Korea. All subjects were asked to submit written consent to participate in the research. Subjects were eligible for the study if they were relatively healthy and consented to participate in the study. Subjects were excluded if they met any of the following exclusion criteria: (i)decreased activities of daily living due to severe physical disease, (ii)metal substances in the body that could affect the fMRI such as dental implants, artificial joints, or pacemakers, (iii) the taking of psychiatric medication or a history of psychiatric disease [14], (iv)severely impaired cognitive

83　function (MMSE score <24) or a clinical diagnosis of dementia, (v)claustrophobia, (vi)
84　contraindications for MRI[15], and (vii)withdrawal of consent to participate in the study.

85　　The protocol was approved by the Institutional Review Board of Kyungpook National
86　University Hospital and was conducted in compliance with research ethics (protocol No. KNUH
87　2015-04-032).

88　*2.2. Screening*

89　　All recruited subjects were initially screened and their demographic and medical information
90　was collected. A questionnaire was conducted to determine the following: age, smoking status,
91　drinking status, regular exercise, past medical history (hypertension, diabetes, dyslipidemia, and
92　history of stroke), and education level, any of which could possibly affect cognitive function.
93　In the first visit to hospital, the subjects' height, weight, waist circumference, blood pressure, and
94　pulse rate were measured. All measurements were performed by the same trained person according
95　to standardized protocols. Body height and weight were measured with a reliable digital
96　height–weight scale. Subjects stood straight without shoes and wore light clothing. Waist
97　circumference (WC) was measured at the middle of the body between the lower line of the ribs and
98　the upper line of the pelvis. Body mass index (BMI) was calculated as weight in kg divided by
99　height squared in meters. Blood pressure and pulse rate were measured using an autonomic blood
100　pressure monitor, with the subjects sitting quietly for 5minutes before measurements. Blood
101　pressure was measured twice, with a brief break in between.

102　　The MMSE was used to assess the baseline cognitive function of the subjects at screening. The
103　MMSE is a 30-point test that evaluates orientation to time (5 points), orientation to place (5 points),
104　memory registration (3 points), attention and calculation (5 points), memory recall (3 points),
105　language (8 points), and copying ability (1 point) [16]

106　*2.3. Randomization and Study Procedure*

107　　The subjects were selected through the screening process and randomly allocated into two
108　groups in a 1:1 ratio: the insect-rearing group and control group. All subjects underwent the same
109　tests during fMRI imaging (see below) at baseline and after 8 weeks.

110　　The oriental garden cricket (*Teleogryllus emma*) was selected as a pet insect for the insect-rearing
111　group, in consultation with insect experts. The reasons for choosing the crickets were, first, the
112　crickets are common in East Asia, and the chirping and appearance of crickets would be familiar to
113　elderly Koreans; second, the connection between crickets and farm life can create nostalgia in the
114　elderly, which can manifest as an affection for insects [17]; third, their small size(26–40mm) means
115　there is little space limitation for insect rearing; and, finally, they can be raised indoors at room
116　temperature and are relatively easy to care for because they are omnivorous [18]. The insect-rearing
117　group received pet insects (4–5 crickets, a uniform distribution of males and females) in a cage and
118　then reared them. Sufficient fodder and all tools necessary for rearing were provided by the
119　researchers. In addition, the researchers provided appropriate training on how to keep the pet
120　insects. Every week, the research assistant confirmed the study subjects' compliance and conducted
121　telephone counseling to encourage the cricket rearing.

122　　The control group was compensated for the possible auditory effects of pet crickets by the
123　receipt of a CD containing meditative music with the natural chirping sound of the crickets, and the
124　research assistant conducted telephone counseling every week to ensure compliance

125   *2.4. fMRI (Functional Magnetic Resonance Imaging)*

126   Blood oxygen level dependent (BOLD) fMRI was acquired on a 3.0-T GE Exite instrument (GE
127   Healthcare) using an 8-channel head coil and a gradient-echo echo-planar imaging (EPI) sequence.
128   T2-weighted MRI images were also acquired. The fMRI protocol used the following acquisition
129   parameters: echo time (TE) = 40 ms, repetition time (TR) = 3000 ms, field of view (FOV) = 22cm,
130   acquisition matrix = 64 × 64, and cross-sectional slice thickness of4mm. All images were acquired
131   parallel to the intercommisural line (anterior commissure–posterior commissure line, ac–pc line).

132   *2.5. Paradigm for the Testing of Working Memory Processing: Wisconsin Card Sorting Test (WCST)*

133   The research assistants thoroughly explained the study protocol to the subjects and a skilled
134   professional conducted the study according to the planned paradigm. The WCST[19] is a known
135   task for testing executive functions. With consideration of the age of the subjects, two conditions of
136   the WCST were utilized: a high level baseline (HLB) and a semi-WCST test. In the HLB version
137   (choose the matching card), when a "matching card" was displayed, the subject was required to
138   select the matching card from the four cards presented on the screen and give the number of the
139   matching card. In the semi-WCST version (with the category by which to select the card informed
140   in advance), a word indicating the category of the card to be selected was presented, followed by a
141   "card" directive. If the "number" was given, the "card" was repeated until the next directive was
142   presented, and a same number card among four cards presented on the screen was selected. There
143   were three categories presented: number, shape, and color.

144   First, the rest condition was measured to show the fixation before and after the task, and then
145   the HLB test and semi-WCST were alternately repeated (Figure1).
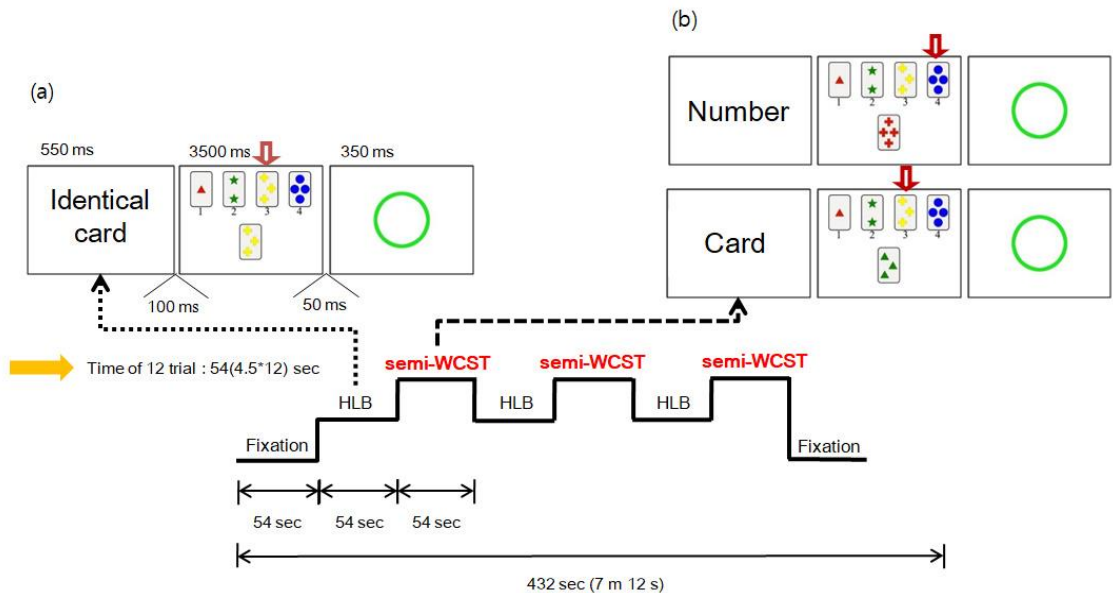

146

147   **Figure 1.**Paradigm for working memory processing. (a)High level baseline (HLB), (b) semi-Wisconsin
148   Card Sorting Test (WCST), and (c) WCST task block design

149   The visual stimuli were shown using standardized software, with the latency and response
150   rates of the correct responses being collected to judge the performance of the subjects by their
151   responses made on the MRI-compatible reply button.

152   *2.6.* Image Analysis

153   The fMRI data image processing and the statistical analysis were performed using SPM5
154   (Welcome   Department   of   Imaging   Neuroscience,   London,   UK;   online   at
155   http://www.fil.ion.ucl.ac.uk) running within MATLAB (MathWorks Inc., Natick, MA). The
156   preprocessing included alignment of the functional images for movement correction, coregistration
157   to the individual structural image, and spatial normalization of all images to the Montreal
158   Neurological Institute (MNI) template and coordinate system. Differences in brain activation
159   among groups were analyzed using random effects analysis after the analysis of individual data.
160   Differences in brain activation when performing the semi-WCST were evaluated using
161   within-group paired *t*-tests. The SPM *t*-score maps were thresholded at $p<0.05$ with family wise
162   error (FWE) correction using Monte Carlo simulations performed using the 3dClustSim program
163   (https://afni.nimh.nih.gov/pub/dist/doc/program_help/3dClustSim.html).

164   *2.7. Stastical Analysis*

165   All subjects had MMSE scores more than 24 at baseline, and the subjects were randomized into two
166   groups in a 1:1 ratio. However, significant deviation of executive function at baseline fMRI was
167   found among the subjects in the insect-rearing group. Therefore, the insect-rearing group was
168   further classified into two groups based on the results of the initial WCST: those with a relatively
169   high score were allocated to insect-rearing group I, and those with a relatively low score to
170   insect-rearing group II. The general characteristics of the subjects were analyzed using ANOVA and
171   Pearson's Chi-square tests. The differences in accuracy between the HLB and semi-WCST tests were
172   statistically compared within and among groups using paired t-tests, ANCOVA, and 2-way (2×2)
173   ANOVA. The fMRI images were analyzed using a flexible factorial design. A p-value of <0.05 was
174   considered to indicate statistical significance. IBMSPSS statistics version25 was used for all
175   statistical analyses.

3. Results

176

177   *3.1. Baseline Characteristics of the Subjects*

178   A total of 44 participants were recruited and screened during the enrollment period. Eight
179   subjects were excluded because they met one or more of the exclusion criteria. The remaining 36
180   subjects were randomized into the insect-rearing (n=20) or control group (n=16). One subject in the
181   insect-rearing group was lost to follow-up. The insect-rearing group was further classified into two
182   groups according to the baseline WCST score: insect-rearing group I (high score, n=13) and
183   insect-rearing group II (low score, n=6; Figure 2).
184   There were no significant differences in the demographic and clinical characteristics among the
185   three groups (table 1). The mean ages were 66.38, 68.31, and 70.67 years for the control,
186   insect-rearing I, and insect-rearing II groups, respectively, while the average MMSE scores at
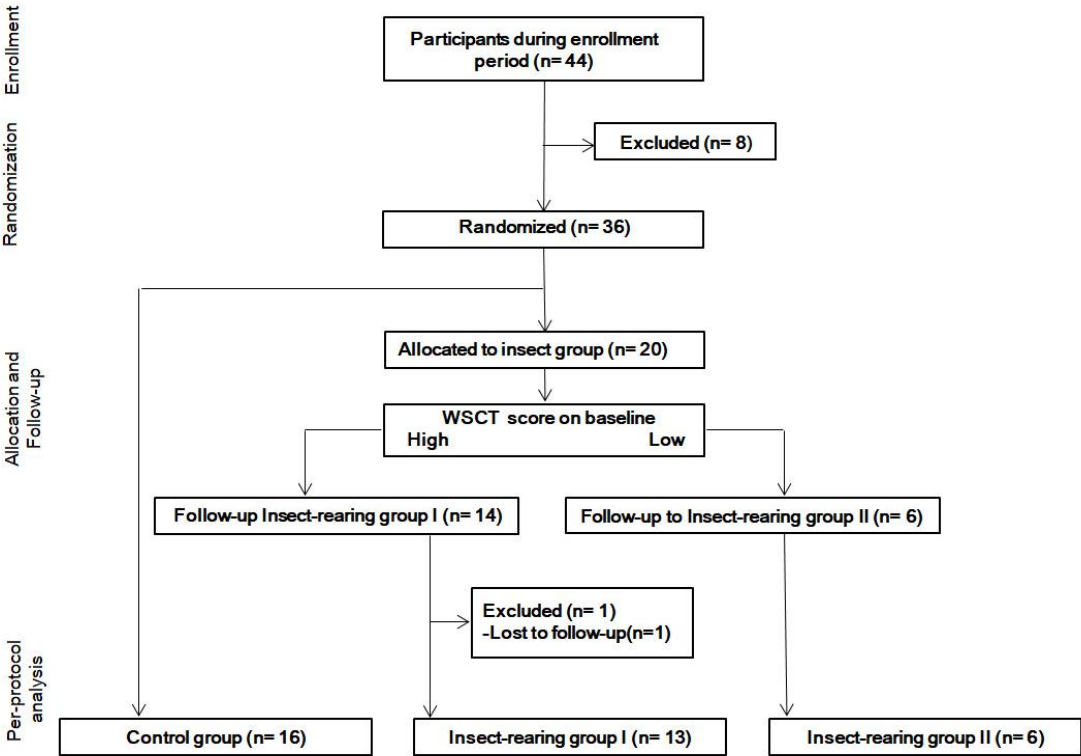187   baseline were 27.94, 27.77, and 27.50.

188

189   **Figure 2.** Flow diagram of the study participants.

190   **Table1.** Baseline characteristics of the subjects in the control, insect-rearing I, and insect-rearing II groups

	Control group (n=16)	Insect-rearing group I (n=13)	Insect-rearing group II (n=6)	p-value*
Age, years	66.38±5.39	68.31±3.75	70.67±3.93	0.15
Height, cm	152.44±5.54	156.16±4.38	153.95±6.57	0.19
Weight, kg	52.48±9.10	65.28 ±24.31	57.60±3.69	0.12
BMI	22.49±3.26	26.93±11.06	24.31± 0.98	0.26
Waist, cm	80.06±8.81	84.19±11.26	85.17±6.43	0.39
Blood pressure, mm Hg				
Systolic	134.25±19.05	137.46±12.89	137.50±21.30	0.86
Diastolic	72.31±13.73	78.15±8.95	71.50±11.06	0.34
Heart rate, beats/min	70.00±9.71	68.85±7.61	68.83±4.02	0.92
Psychometric tests				
MMSE(cognitive function)	27.94±1.98	27.77±1.74	27.50±1.64	0.88
Smoking status				
Non- or ex-smoker	15(93.8)	13(100.0)	6(100.0)	1.000‡
Current smoker	1(6.3)	0(0.0)	0(0.0)	
Alcohol consumption				

None	12(75.0)	12(92.3)	5(83.3)	0.613[†]
Any	4(25.0)	1(7.7)	1(16.7)	
Exercise				
None	15(93.8)	12(92.3)	5(83.3)	0.762[†]
Regular	1(6.3)	1(7.7)	1(16.7)	
Education				
≤Elementary school	8 (50.0)	8 (61.5)	5 (83.3)	0.125[†]
≥Middle school	8 (50.0)	5 (38.5)	1 (16.7)	
Clinically diagnosed underlying disease				
Hypertension	6(37.5)	7(53.8)	1(16.7)	0.317[†]
Diabetes	1(6.3)	2(15.4)	0(0.0)	0.762[†]
Dyslipidemia	5(31.3)	9(69.2)	2(33.3)	0.113[†]
CVA	0(0.0)	1(7.7)	0(0.0)	0.543[†]

191   The data are presented as mean ± standard deviation or number (%).

192   [*]The two groups were compared using independent *t*-tests or Pearson's χ2 test except when indicated

193   otherwise.

194   [†]Fisher's exact test.

195   *3.2. WCST Response Data Results*

196   At baseline, the average HLB–semi-WCST accuracies were 30.21%, 35.68%, and 22.22% in the

197   control, insect-rearing I, and insect-rearing II groups, respectively, with there being no significant

198   difference among groups. After 8 weeks, the corresponding average values were 25.00%, 29.70%,

199   and 44.44%, and the difference was close to being statistically significant (0.06). In insect-rearing

200   group II, the mean accuracy of the difference between the HLB and semi-WCST tests improved

201   from 22.22% at baseline to 44.44% after 8 weeks, showing a statistically significant improvement

202   (p=0.01, Figure 3, Table 2). There were no other significant differences in the intra group

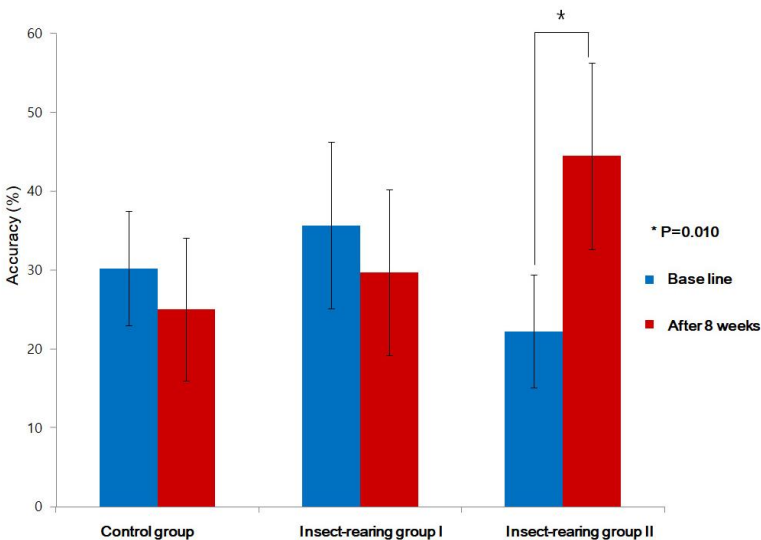203   HLB–semi-WCST test accuracies between the baseline test and that after 8 weeks.

204

205   **Figure 3.**High level baseline (HLB)–semi-Wisconsin Card Sorting Test (WCST) accuracy, paired *t*-test

206   (p=0.010).

207   **Table2.** WCST response pre- and post-intervention among the groups

		Control group	Insect-rearing group I	Insect-rearing group II	p-value†
Accuracy (%)	**Baseline**	30.21±13.64	35.68±17.49	22.22±6.80	0.180
	After 8 weeks	25.00±16.43	29.70±17.36	44.44±11.25	0.060
p-value*		0.329	0.319	0.010	

208   *Paired *t*-test and † ANOVA of HLB−semi-WCST accuracy (%).WCST=Wisconsin Card Sorting

209   *3.3. fMRI Data Analysis Results*

210   The brain activation maps calculated for the acquisitions before and after 8 weeks are shown in
211   Table 3 and Figure 4 for the control and insect-rearing groups. There was no significant group
212   difference in brain activation area (paired *t*-test) during the fMRI test between the acquisitions
213   before and after the intervention in either the control group or insect-rearing group I. However, in
214   insect-rearing group II, the brain activation areas during the WCST test were different before and
215   after the pet insect-assisted therapy. In this group, the paired *t*-tests analyzing the differences before
216   and after pet insect-assisted therapy showed peak*t*-scores of 16.29 in the left mid frontal area, 8.53
217   in the left superior medial frontal gyrus, 5.51 in the right inferior frontal gyrus, 8.96 in the right
218   putamen, 8.88 in the left insula, 8.88 and 7.88in the left and right hippocampi, and 17.61and 8.01in
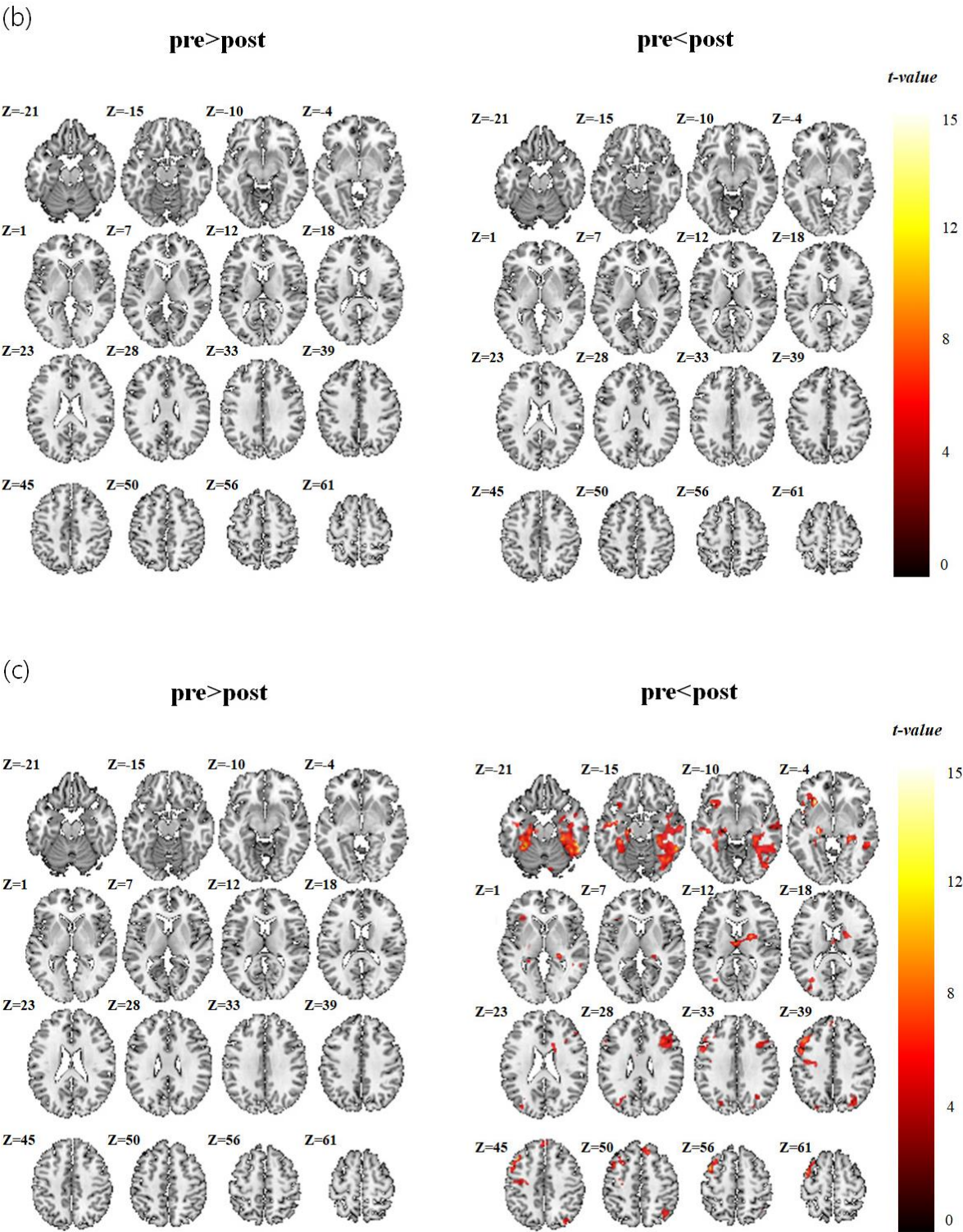219   the left and right fusiform gyri.

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221

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(b)

(c)
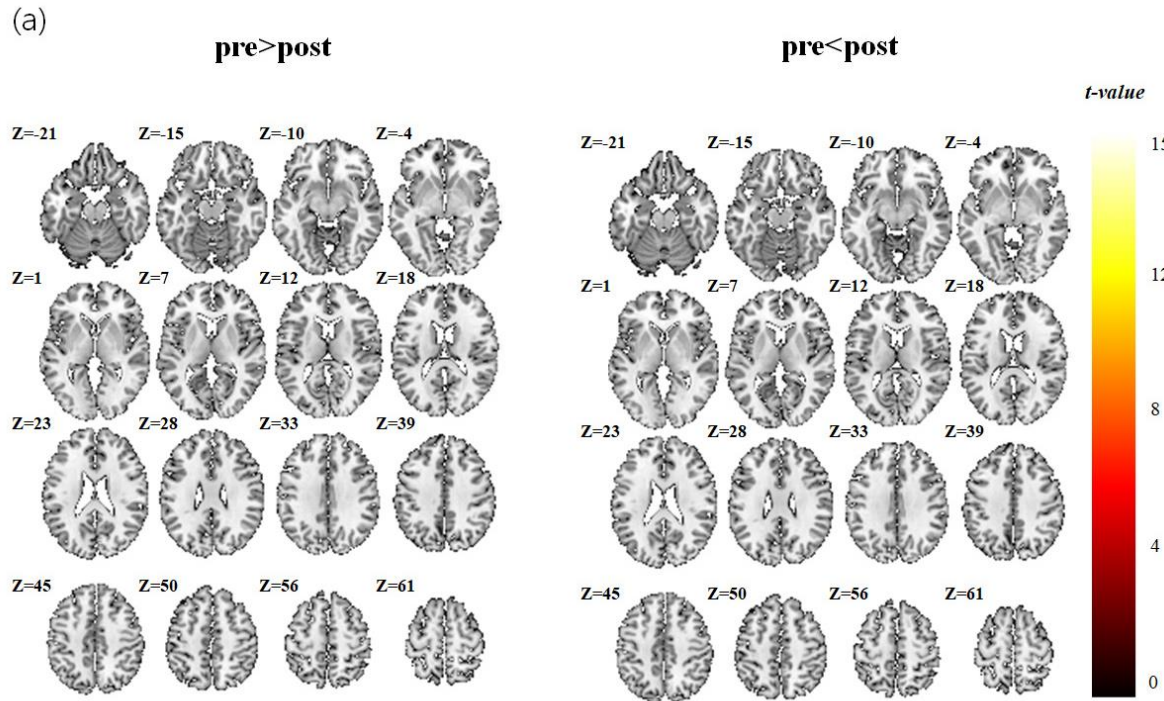
Figure 4.Brain activation maps for paired *t*-test analysis (p<0.05, with FWE correction using Monte Carlo simulation by 3dClusSim and a minimum cluster size of 163). (a) Control group, (b) Insect-rearing group I, (c) Insect-rearing group II

230   **Table 3.Brain activation map scores for paired t-test analysis of insect-rearing group**

Contrast	Region		Cluster size	Coordinates (mm)			Peak T
				x	y	z	
semi – HLB	Middle frontal gyrus	L	544	-46	22	44	16.29
	Superior medial frontal gyrus	L	97	-2	48	40	8.53
	Precentral gyrus	L	79	-42	-10	36	5.07
	Superior parietal lobule	R	-100	38	-60	50	4.77
	Inferior frontal gyrus	R	158	38	16	34	5.51
	Precuneus	L	20	-24	-82	36	4.31
		R	110	28	-80	38	4.58
	Putamen	R	57	22	0	12	8.96
	Thalamus	L	60	-4	-12	10	6.88
		R	49	4	-12	12	5.03
	Insula	L	173	-32	14	-14	8.88
	Inferior orbito-frontal gyrus	L	147	-36	20	-14	5.26
	Hippocampus	L	133	-20	-22	-14	8.88
		R	236	24	-34	-4	7.88
	Fusiform gyrus	L	572	-36	-36	-20	17.61
		R	1072	28	-30	-20	8.01

231   p <0.05, with FWE correction using Monte Carlo simulation by 3dClusSim and a minimum cluster size of 163.
232   The x, y, and z coordinates are the Montreal Neurological Institute (MNI) coordinates. Cluster size is the
233   number of voxels activated in a regional cluster. L = left, R =right, T is the *t*-values of the supra threshold
234   voxels.

235   **4. Discussion**

236   This study was a randomized single-arm controlled trial that investigated the effects of rearing
237   pet insects on cognitive function in elderly women. The effects on cognitive function were assessed
238   using fMRI. The insect-rearing group II, with a relatively low average baseline executive function,
239   showed significant differences in both WCST response and fMRI results after pet insect-assisted
240   therapy. This result showed that the rearing of pet insects had a beneficial effect on executive
241   function in community-dwelling elderly women with low cognitive function.
242   fMRI has frequently been used in neuropsychological studies of memory. Memory can be
243   divided into short-term memory (working memory) and long-term memory, and fMRI primarily
244   shows working memory according to the functioning of the frontal lobe. It was reported that the

245   left frontal lobe encodes working memory and retrieves working memory from the right frontal
246   lobe [20]. Frontal cortex activation during the performance of memory tasks was demonstrated
247   using fMRI. fMRI can also be used to distinguish activation in three areas of the frontal cortex:
248   anterior frontal cortex (AFC), dorsolateral frontal cortex (DLFC), and ventrolateral frontal cortex
249   (VLFC).The DLFC and VLFC are located above and below the inferior frontal gyrus, respectively,
250   while the AFC is located in front of the inferior frontal gyrus. There are no clear boundaries but the
251   regions can be divided into Brodmann areas, with the VLFC including Brodmann areas 44, 45, and
252   47; the DLFC, areas 9 and26; and the AFC, areas 8 and 10. AFC, VLFC, and DLFC are distinguished
253   according to their activation levels in different tasks:(i) updating and maintaining the contents of
254   working memory, (ii) selecting, manipulating, and monitoring the contents of working memory, and
255   (iii) selecting process, target, and sub-goal. These three functions closely match the patterns of VLFC,
256   DLFC, and AFC activation, respectively [21]. The prefrontal cortex is included in the frontal lobe,
257   and an advanced study has shown that the prefrontal cortex is associated with executive function
258   [22].

259   The WCST is a multifaceted test requiring the use of a distributed brain network, and task
260   execution ability may be impaired by various factors. Some of these are related to frontal lobe
261   function [23]; most neuroimaging studies on WCST have reported a significant increase in
262   metabolic or neural activity in the frontal or prefrontal cortical region. In addition, in most studies,
263   an increase in brain activation has been found in the dorsolateral prefrontal cortex(DPFC), and in
264   some studies it has also been found in the ventromedial prefrontal cortex(VPFC) [19]. Functionally,
265   the lateral prefrontal cortex (LPFC)is involved in organizing, keeping, and manipulating
266   information in the short term, depending on the type of cognitive task [24].

267   In working memory tasks, the brain areas activated include the left and right Dorsolateral
268   prefrontal cortex, (DLPFC), left Ventrolateral prefrontal cortex(VLPFC), premotor cortex, right
269   frontal pole, bilateral inferior parietal lobules, right insula, right temporal gyrus, and a subcortical
270   region at the junction of the left thalamus, caudate, and lenticular nucleus. These brain areas are
271   also functionally connected for performing working memory tasks [25], with working memory
272   being a main cognitive function [14]. In this study, insect-rearing group II showed enhanced
273   activation signals in the left medullary frontal area, right DLPFC, parietal cortex, left insula, and
274   both hippocampi after the rearing of pet insects. During the WCST, the brain showed greater
275   activation for executive tasks involving the frontal (especially DLPFC) and parietal cortex and the
276   hippocampi, which are working memory areas. This indicates that the executive ability required to
277   perform the WSCT task was improved, and that the rearing of insects had a positive effect,
278   improving cognitive function.

279   Insect-rearing group II showed a relatively low score at baseline, which may be considered to
280   be the result of a low understanding of how to perform the WCST. However, subjects did not
281   significantly differ among groups in their MMSE sore, with all groups showing a normal range. A
282   previous study suggested that the delayed recall items of the MMSE are an index of working
283   memory capacity [26]. We made no evaluation of the sub-content items of the MMSE, and even if
284   the MMSE scores were within normal ranges, there may still have been cognitive impairment with
285   false-negatives. The most common impairments of cognitive function in patients with false-negative
286   results are memory and frontal lobe-executive functions [27]. Advanced studies have already
287   indicated that MMSE is insufficient to evaluate the executive function of the frontal lobe [28, 29].
288   The MMSE evaluation showed that all subjects were in the normal cognitive state, but we can infer
289   that the group with low WCST scores had relatively low cognitive functioning.

290   However, there was no significant difference in the accuracy rate and brain activation in the
291   WCST response data among the three groups. Furthermore, when the differences between the
292   WCST response data acquired before and after the intervention were compared within groups,
293   there was no significant change in the control or insect-rearing I group. Previous studies have
294   reported that AAT could improve cognitive function in elderly subjects [5], and a significant
295   improvement in cognitive function score was found in a previous study [30] using pet insects in
296   Korea. Based on these previous studies, we expected that fMRI-detected cognitive function would
297   be improved in this study. The reasons for the absence of significant differences among the three
298   groups after 8 weeks of pet insect-assisted therapy may include the following. First, the number of
299   subjects was small, which may have resulted in the failure to find statistically significant differences
300   in analysis conducted within groups after randomized classification. Second, all the subjects were
301   elderly women and most of them (58%) had an educational level below that of elementary school
302   graduation. Previous studies [31, 32] have shown that age, sex, and education level are significantly
303   associated with cognitive and neuropsychological test results. However, the subjects were limited
304   to elderly women, and both age and education level showed no significant difference among the
305   groups. Third, the subjects were limited to those who had favorable cognitive functioning with a
306   score of 24 points or more on the MMSE. As the subjects had a normal range of cognitive function at
307   baseline, there is the possibility that a noticeable improvement in cognitive function was not
308   detectable after a study period of 8 weeks. However, it is difficult for subjects with relatively low
309   cognitive function to conduct the complex WCST and to receive education about the protocol before
310   the test; a high MMSE score was an inevitable criterion because of the study design characteristics.
311   Fourth, the study period of 8 weeks was relatively short and may have been insufficient to see a
312   change in cognitive function. However, this was an inevitable limitation because oriental garden
313   crickets only live for up to 8 weeks. A previous study [33] on elderly subjects in a nursing home
314   conducted AAT for 12 months. The mean Mental Function Impairment Scale (MENFIS) was
315   reduced after 6 months, which means that cognitive function improved after 6 months. In another
316   study [34] on elderly Alzheimer's disease patients, cognitive function improvement measured by
317   the MMSE was observed in a group who underwent AAT for 6 months. We suggest that to achieve
318   meaningful improvement in cognitive function using insect-assisted therapy, the study should be
319   conducted for a longer period.

320   This study has a few limitations. First, all subjects were community-dwelling elderly Asian
321   women living in a single Korean city. Thus, it may be impossible to generalize the results to other
322   ethnicities or younger populations. Second, the period of study was 8 weeks, which was relatively
323   short. As mentioned above, this short duration may have been insufficient to show improvement in
324   cognitive function. Third, the reliability of the WCST score may have been lower at baseline than at
325   8 weeks. To conduct the WCST, the method was explained in advance, but the elderly subjects may
326   have found it difficult to understand. Also, there is the possibility of a learning effect because the
327   same test was performed twice.

328   Despite these limitations, this study also has its strengths. First, it was a randomized controlled
329   design. Second, it is the first study to use fMRI to investigate the effect of rearing pet insects on
330   cognitive function. Although a previous study [30] investigated the clinical effects of rearing pet
331   insects, this study used a more objective approach by analyzing brain imaging data. Third, every
332   week, the research assistant confirmed compliance with the rearing of the pet insects and conducted

333   telephone counseling to encourage the practice. Fourth, the precise effects of rearing pet insects on
334   cognitive improvement were determined by performing WCST and fMRI imaging together, and
335   these are well known to be reliable and valid tools. Finally, as mentioned above, the restricted
336   subject diversity could be a strength because it should minimize the confounding effects of
337   variables such as age, sex, residential area, and comorbid diseases.

338   In conclusion, the rearing of pet insects was found to have a positive effect on improving
339   executive function and performance in elderly women. Rearing insects is cost-effective, easy,
340   occupies less space than the raising of larger animals, and can be usefully used as a type of AAT
341   that is non-pharmacologically based and noninvasive. Further larger studies of pet insects with
342   longer study periods are warranted.

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