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Original Article

Recreational Music-Making Modulates Immunological Responses and Mood States in Older Adults

Masahiro Koyama[1, 2], Masatada Wachi[1], Masanori Utsuyama[1, 4], Barry Bittman[3], Katsuiku Hirokawa[1, 4] and Masanobu Kitagawa[1]

1) Department of Comprehensive Pathology, Tokyo Medical and Dental University Graduate School, Tokyo, Japan
2) Center for Advanced Sound Technologies, Yamaha Corporation, Hamamatsu, Japan
3) Meadville Medical Center, Mind-Body Wellness Center, Meadville, PA, U.S.A
4) Institute for Health and Life Science, Tokyo Japan

Given that previous studies have shown that recreational music-making has benefits for younger individuals, we explored two questions. 1. Could a recreational music-making protocol improve mood and modulate immunological responses in a direction opposite to that associated with chronic stress in older adults? 2. Would the protocol affect older and younger participants differently? Two groups of volunteers demarcated at age 65 years underwent identical one-hour recreational music-making interventions. Pre-and post-intervention data were collected using blood samples and mood state questionnaires. Data from 27 older and 27 younger volunteers were analyzed for cytokine production levels, natural killer cell activity, plasma catecholamines, and numbers of T cells, T cell subsets, B cells, and natural killer cells. Exercise expenditure was also recorded. In the older group, we found significant increases in the number of lymphocytes, T cells, $CD4^+$ T cells, memory T cells, and production of interferon- γ and interleukin-6. In the younger group, modulation was non-significant. Worthy of note was the specific immunological changes in the direction opposite to that expected with chronic stress in the older group. The increase in Th1 cytokine IFN-γ and unchanged Th2 cytokine IL-4 and IL-10 levels in the older group suggests a shift to a Th1-dominant status, a shift opposite to that expected with stress. However, the immunological changes were not statistically different between the two groups. Mood states improved in both groups, but were also not statistically different between groups. Although no statistically significant difference was found between the two age groups, the improvement in immunological profile and mood states in the older group and the low level of energy required for participation suggest this music-making protocol has potential as a health improvement strategy for older individuals.

Key words : recreational music-making, elderly, psychological stress, cellular immunity

Corresponding Author : Masahiro Koyama
Department of Comprehensive Pathology Graduate School Tokyo Medical and Dental University 1-5-45, Yushima, Bunkyo-ku, Tokyo, Japan 113-8519
Telephone : +81-3-5803-5175 Facsimile : +81-3-5803-0123
E-mail : masahiro_koyama@gmx. yamaha. com
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Introduction

Promoting the health and welfare of the elderly is especially critical in rapidly aging nations such as Japan. By 2050, people over 65 years of age will make up 35% of the population, making Japan the most aged nation in the world. The economic and social impact of an aging society will also be felt by many other countries in the first half of this century. Based upon scientific evidence suggesting that senescent changes in nervous, endocrine, and immunological systems underlie reduced tolerance to stress in the elderly [1], the development of health management strategies that can counter these challenges is essential. This is especially crucial since later in life many people experience

increased stress as a result of physical, social, and economic challenges such as death of family members or reduced income.

The benefits of recreational music-making (RMM) protocols that provide opportunities for self-expression without requiring musical talent or experience have been reported by Bittman et al.[2,3,4,5] Increased natural killer (NK) cell activity and dehydroepiandrosterone-to-cortisol ratios were reported in association with a specific group drumming protocol.[2] Reduced burnout and improved mood dimensions have also been documented in a group drumming protocol for nursing students.[4] The effectiveness of a similar RMM group drumming intervention as a means of reducing stress in the workplace has also been examined by Wachi et al.[6] on healthy male Japanese corporate employees younger than 60 years of age. The study demonstrated that natural killer cell activity was modulated toward normal levels and the expression of stress-induced cytokine interleukin (IL) 10 mRNA was reduced after the RMM intervention. These studies by Bittman et al. and Wachi et al. investigated the effects of RMM on the immunological profile and mood states of young and middle-aged adults, but not of older people.

Research suggests that reductions in the number of peripheral blood T cells, B cells, $CD4^+$ T cells are associated with chronic stress.[7,8,9] Studies also suggest that the immune system becomes less effective as people age.[1,10,11] Hirokawa et al. reported that with age, the nervous, endocrine, and immune systems undergo change, rendering older people less able to respond biologically to serious stressful situations.[1,11]

In consideration of the reported benefits of RMM and the findings on the effects of stress and senescence on immune responses, we asked two questions. 1. Could a recreational music-making protocol improve mood and modulate immunological responses in a direction opposite to that associated with chronic stress in older adults? 2. Would the protocol affect older and younger participants differently?

Therefore we collected and analyzed data on immunological responses and mood states of two groups of healthy Japanese adults—one group younger than and the other group older than age 65. Specifically, we examined immunological cell counts, NK cell activity, catecholamine levels, and cytokine production levels in samples taken before and after a specific RMM protocol. We also included an assessment of energy expenditure to determine whether physical activity affected the results.

Material and Methods

Approval of the experimental protocol and informed consent form

The experimental protocol of this study was reviewed and approved by Tokyo Medical and Dental University's Ethics Committee for Human Studies, Tokyo, Japan (application number 380). The informed consent form used was also approved by the Ethics Committee.

Recruitment of participants

A total of 63 people were selected for the study, 30 of whom were in the older group and 33 in the younger group. The breakpoint age for the two groups was 65 years. In the recruiting process, it was explained that during the four-hour session subjects would be required to participate in a one-hour group drumming intervention, complete two questionnaires, and undergo blood sampling before and after the intervention. It was explained that each participant would receive a prepaid shopping card worth 5000 yen on the day of participation as well as a subsequent report on their immunological status. Subject selection was consistent with the exclusionary criteria and precautions set forth in accordance with the study by Bittman et al.[2] The exclusionary criteria included active medical illnesses, infections, receiving treatment for a medical problem, a history of heart and lung disease, smoking, self-reported hearing loss, pregnancy, listening to drumming music on a regular basis, and participation in drumming sessions during the past six months. Two or three days prior to the session, participants were contacted by telephone and reminded to refrain from engaging in aerobic activity 24 hours prior to the session, and from consuming food or alcohol two hours prior to the session.

Experimental Design

To ensure a personalized experience, both groups were divided into three subgroups of nine to 12 volunteers. All six subgroups underwent identical one-hour RMM sessions held on Tuesdays between 1 : 15 pm and 5 : 30 pm.

Clinical Methods

Participants were asked to present to the Care Plaza Kotobuki, a small-scale multi-functional residential

Table 1. Recreational music-making group drumming protocol.

Building blocks	Time*	Outline
1. Introducing the program	1	Putting participants at ease.
2. Breathing	1	Focusing on breathing and relaxing.
3. Breaking the ice	12	Passing a shaker in a circle, finally resulting in someone dropping the shaker, and eliciting a round of laughter.
4. The A-B-Cs of drumming	11	Introducing the basics of drumming.
5. Rhythmic naming	7	Each person plays their name on their instrument. The group echoes it.
6. Entrainment building	15	Facilitator leads with a basic rhythm, participants join in one by one, and the group is led to produce a more complex rhythm.
7. Inspirational beats	4	Inspiring participants to create "the groove".
8. Resonance within	3	Participants express their feelings and stress on their instrument.
9. Guided imagery drumming	6	Drumming to an image, e.g. participants are invited to go on a canoe journey down an ethereal stream echoing a naturally calming symphony.
10. The finale	3	Focusing on breathing and the reverberation of the drum beat, and asking each person to describe verbally or rhythmically what the drum circle meant for them.

* Mean duration in minutes.

care centre for the elderly in Abiko city 75 minutes prior to the actual intervention. After a verbal explanation presented by a physician, all participants read and signed the informed consent and completed checklists for the exclusionary criteria and precautions. Volunteers were then fitted with SenseWear PRO_2 Armbands (BodyMedia Inc, PA, USA) [12,13,14] on their right upper arm to obtain energy expenditure data during the intervention. Participants then completed the Japanese edition of the Profile of Mood States questionnaire (POMS, Kaneko Shobo, Tokyo, Japan).[15] A peripheral blood sample (maximum 22 ml) was subsequently drawn in accordance with standard laboratory technique. All blood samples were obtained in less than 9 minutes by an experienced team of nurses. Participants were then taken to a room with chairs arranged in a circle. They participated in a RMM group drumming session that lasted for approximately one hour. All RMM sessions were led by an experienced facilitator who had been trained in accordance with the HealthRHYTHMS™ *Group Empowerment Drumming* protocol (Table 1). The same facilitator led all RMM sessions. The primary objective was to promote the enjoyment and well-being of the participants by eliciting laughter, self-expression, and a sense of togetherness. Immediately after the RMM session, blood sampling was undertaken using the procedure noted above. Participants then completed a second POMS questionnaire after which the armbands were removed. Subsequently, participants received a report on their immunological status compiled by the Institute for Health and Life Science (Tokyo, Japan) by mail.

Laboratory Studies

Blood specimens

Two milliliters of blood was drawn into a tube containing ethylenediaminetetraacetic acid for hematological analysis. Five milliliters of blood was drawn into a preparation tube for NK cell activity analysis. Seven milliliters of blood was drawn into a tube containing ethylenediaminetetraacetic acid for catecholamine analysis. Eight milliliters of blood was drawn into a preparation tube for peripheral blood mononuclear cells (PBMC).

Hematological analysis

Peripheral blood samples were evaluated using an automatic hemocytometer (PENTA80, Horiba, Kyoto, Japan).

Natural killer cell activity

NK cell activity in PBMC was measured by chromium-51 release assay using K562 cells as targets with an effector-to-target ratio of 20 : 1 (SRL Inc. Tokyo, Japan).[6]

Catecholamines

Plasma separation was performed immediately after

blood sampling. The samples were stored at −80℃. Subsequently, the samples were deproteinized (centrifuged after addition of 5% perchloric acid solution and stirring followed by addition of 1.5mol/L sodium acetate and stirring). The supernatant was then injected into a high-performance liquid chromatography system for the determination of adrenaline, noradrenaline, and dopamine.[16] The HPLC system consisted of two precolumns (TSK precolumn CA1 and CA2, Tosoh Co., Tokyo, Japan), an analytical column (Wakosil-II 5C18RS, Wako Co., Osaka, Japan), and a fluorescence detector (L-7480, Hitachi Co., Tokyo, Japan). NK cell activity and catecholamine assays were performed by SRL Inc.

Peripheral blood mononuclear cells

PBMC were analyzed by flow cytometry (two or three colors) with fluorescein-labeled monoclonal antibodies (mAb) using a flow cytometer (FACScan, Becton Dickinson, NJ, USA).[17] The following mAb were used : Fluorescein isothiocyanate (FITC)-conjugated anti-CD4 mAb, FITC-conjugated anti-CD20 mAb, FITC-con jugated anti-CD16 mAb, Phycoerythrin (RD1)-conjugated anti-CD3 mAb, RD1-conjugated anti-CD8 mAb, Phycoerythrin-Texas Red (ECD)-conjugated anti-CD45RA mAb, ECD-conjugated anti-CD3 mAb, Phycoerythrin (PE)-conjugated anti-CD56 mAb, PE-conjugated anti-CD4 mAb, and PE-conjugated anti-CD8 mAb. All mAb were obtained from Beckman Coulter and were used in the following combinations (analyzed cells are shown in parentheses) : CD3-RD1/CD20-FITC (T cells, B cells), CD4-FITC/CD8-RD1/CD45RA-ECD ($CD4^+$ T cells, $CD8^+$ T cells, naive T cells, memory T cells), and CD56-PE/CD16-FIFC (NK cells). Data obtained were converted to number of cells per μ L of peripheral blood.

Assessment of cytokine production

Anti-CD3 mAb was immobilized on a 24-well plate in which 1×10^6 PBMC were cultured for 48 hours. Production levels of interferon gamma (IFN-γ), IL-2, IL-4, IL-6, and IL-10 were assessed using ELISA immunoassay kit (Biosource International, CA, USA).[11,18]

Mood states

The questionnaire filled out by participants before and after each intervention included six dimensions : tension/anxiety, depression/dejection, anger/hostility, vigor/activity, fatigue/inertia, confusion/bewilderment.[18] The raw scores for these dimensions were transformed into T-scores corresponding to age. The T-scores were calculated using the following formula : T-score = 50+10×(raw score of subject−average score)/standard deviation. The average score and standard deviation were taken from the data classified by age and sex in the Japanese version of the POMS manual.[15] The total mood disturbance score for each participant was calculated by subtracting the score for vigor/activity from the sum of the scores for the five other mood dimensions.[6]

Exercise expenditure

The SenseWear PRO_2 Armband used to record energy expenditure data is equipped with a 2-axis accelerometer, heat flux sensor, galvanic skin response sensor, skin temperature sensor, and a near-body ambient temperature sensor that gathers and analyzes physiologic data. Inner View Research software 4.1, (BodyMedia Inc, PA, USA), the dedicated SenseWear PRO_2 Armband software, was used to calculate kilocalories consumed during the one-hour intervention.

Data Exclusion

The data of three older and six younger subjects were excluded from the study. The reasons for rejection included unsuccessful blood sampling, inadequate responses to the health questionnaire, and failure to meet biological health criteria (Table 2). All analyses were therefore based on data from 27 older volunteers (15 women, 12 men) aged 66 to 78 years (mean = 70.3, SD = 2.9) and 27 younger volunteers (19 women, 8 men) aged 19 to 46 years (mean = 27.9, SD = 8.4). Biochemical tests were performed by Sanritsu (Chiba, Japan) to determine the health status of all participants.

Statistical Analysis

In view of the diverse scaling of the measurements (white blood cell count, NK cell activity percentages, and cytokines production levels), pre- and post-intervention values for each individual were transformed into normalized measures of change referred to as “change” :

$\log_e$ [(post-intervention value)/(pre-intervention value)]

The formula above is equivalent to the rate of change below in the first order approximation.

Rate of change=[(post-intervention value−pre-intervention value)/(pre-intervention value)]

“Group difference” refers to “mean change for the older group” minus “mean change for the younger group.”

Table 2. Biochemical criteria used to assess subjects' health.

Laboratory value	Normal range
White blood cells	3500－9300 /μL
Hemoglobin	men 10－18 g/dL ; women 10－16 g/dL
Hematocrit	men 35－53% ; women 32－47%
AST	5－70 IU/L
ALT	5－75 IU/L
GGT	< 90 IU/L
Total cholesterol	130－300 mg/dL
HDL cholesterol	40－105 mg/dL
LDL cholesterol	55－210 mg/dL
Uric acid	men 3.8－7.0 mg/dL ; women 2.4－7.0 mg/dL
Creatinine	men 0.4－1.14 mg/dL ; women 0.31－0.88 mg/dL
Total protein	6.7－8.3 g/dL
Albumin	3.8－5.3 g/dL
Urea nitrogen	6－21 mg/dL
Glucose	70－180 mg/dL
Glycoalbumin	10－20%
C-reactive protein	< 0.5 mg/dL

AST denotes aspartate aminotransferase ; ALT, alanine aminotransferase ; GGT, γ-glutamyl transpeptidase ; HDL, high density lipoprotein ; LDL, low density lipoprotein.

Statistical significance of mean normalized changes was evaluated using the two-tailed paired t-test for both the older and younger group. A mean change greater than zero indicates that on average the post-intervention values increased after RMM intervention. The statistical significance of group differences was analyzed using the two-tailed student's t test for assumptions of homoscedasticity and two-tailed Welch's t test for non-homoscedasticity. If the normality of the distribution of the change was rejected by Shapiro-Wilk test, the significance was corroborated using nonparametric tests. A 0.05 significance level was adjusted for multiple comparisons by Holm's method.[20]

Correlations between variables were evaluated using Pearson product-moment correlation analysis. When the results were statistically significant, a simple linear regression analysis was done. Outliers that had a substantial effect on regression coefficients were indicated and excluded from the regression analyses. Cook's distance which measures the influence of sample values on regression coefficients was calculated, and values greater than 4/(sample size) were considered to be outliers.[6]

SPSS 14.0 for Windows (SPSS Inc., Chicago, IL, U.S.A.) was used for performing statistical analysis.

Results

The mean values of the pre- and post-intervention laboratory data and mood states are given with results on group differences in Table 3. Compared with the younger group, the pre-intervention mean values of the older group were significantly higher for ratio of $CD4^+$ T cells to $CD8^+$ T cells, memory T cell count, and IL-2 production. By contrast, the younger group demonstrated higher mean values for IL-4 and IL-10 production. No significant group difference was confirmed for the pre-intervention mean POMS score.

Mood states after RMM

Significant mood state improvements were noted in both the older and younger groups for tension/anxiety, depression/dejection, anger/hostility, and total mood disturbance by paired t tests (Table 4, Fig. 1). Although in the younger group, scores for fatigue/inertia and confusion/bewilderment decreased significantly indicating mood state improvements, such was not noted in the older group. No significant group difference was found.

Differences between sexes for each group were evaluated using normalized pre- and post-intervention changes. No significant difference between the sexes was confirmed in the older group. However a significant difference between the sexes in the younger group was confirmed for fatigue/inertia ($p = 0.004$).

Changes in peripheral blood values

Pre- and post-intervention changes in peripheral blood values for the two groups were analyzed using the paired t test. Group differences were analyzed by independent t tests (Table 5). Peripheral blood values for the older group showed significant increases in noradrenaline levels, lymphocyte count, T cell counts, $CD\ 4^+$ T cell counts, memory T cell counts, IFN-γ, and IL-6 (Fig. 2). No significant difference was confirmed for the peripheral blood values of the younger group. Values that did not change significantly for both groups included adrenaline, dopamine, NK cell activity, white blood cells, neutrophil counts, B cells, $CD8^+$ T cell counts, ratio of $CD4^+$ T cells to $CD8^+$ T cells, naive T cells, ratio of naive T cells to memory T cells, NK cell counts, IL-2, IL-4, and IL-10. Group difference was significant for IL-6 production ($p < 0.001$).

Table 3. Mean values of biological data and mood states before and after intervention (original scale).

		Older group					Younger group				
		pre-intervention			post-intervention		pre-intervention			post-intervention	
Parameter		n	mean	± SD	mean	± SD	n	mean	± SD	mean	± SD
Biological parameters											
Adrenaline, pg/mL	*	27	48.0	± 29.4	44.2	± 21.5	27	44.5	± 20.3	47.1	± 20.4
Noradrenaline, pg/mL		27	526.1	± 194.6	636.9	± 275.1	27	427.5	± 180.3	463.5	± 164.6
Dopamine, pg/mL	*	27	21.6	± 8.7	21.9	± 7.0	27	17.5	± 6.9	18.3	± 6.3
NK cell activity, % lysis	*	27	33.6	± 15.0	35.4	± 14.6	27	25.6	± 11.1	24.7	± 10.5
White blood cell count, /μL		27	5511	± 1073	5815	± 1011	27	6137	± 1275	6259	± 1329
Neutrophil count, /μL	*	27	2973	± 846	3059	± 730	27	3636	± 1007	3731	± 1031
Lymphocyte count, /μL		27	2093	± 470	2287	± 563	27	1958	± 376	1997	± 383
T cell count, /μL		27	1486	± 359	1629	± 459	27	1431	± 305	1485	± 321
B cell count, /μL	*	27	123.8	± 65.1	144.9	± 80.5	27	170.6	± 69.3	176.0	± 50.5
$CD4^+$ T cell count, /μL	†	26	980.1	± 302.3	1078.3	± 370.9	27	787.3	± 170.2	805.6	± 190.0
$CD8^+$ T cell count, /μL		26	397.3	± 166.3	420.7	± 177.6	27	518.0	± 185.4	534.3	± 168.6
CD4/CD8 ratio	‡†	26	2.862	± 1.407	2.963	± 1.472	27	1.648	± 0.499	1.623	± 0.539
Naive T cell count, /μL	†	26	409.2	± 229.1	455.8	± 278.7	27	374.3	± 127.3	372.3	± 140.5
Memory T cell count, /μL	‡†	26	571.0	± 185.1	622.5	± 209.7	27	413.1	± 89.3	433.3	± 85.3
Naive/memory ratio	*	26	0.786	± 0.537	0.799	± 0.56	27	0.937	± 0.37	0.869	± 0.311
NK cell count, /μL	*	27	440.7	± 200.8	462.1	± 224.5	27	293.5	± 180.0	266.7	± 141.8
IFN-γ production, pg/mL	*	26	11317	± 6106	16948	± 9136	25	13130	± 11763	14842	± 11569
IL-2 production, pg/mL	‡*	26	466.6	± 360.9	519.9	± 468.0	25	188.4	± 61.1	243.2	± 225.1
IL-4 production, pg/mL	‡	25	17.28	± 8.60	17.54	± 9.21	25	39.71	± 9.96	37.9	± 12.11
IL-6 production, pg/mL	*	26	6847	± 4684	11222	± 6039	25	26496	± 49046	17829	± 22118
IL-10 production, pg/mL	‡*	26	300.3	± 231.7	297.7	± 229.2	25	520.2	± 273.4	502.9	± 356.4
Mood states											
Tension/anxiety		26	49.4	± 8.3	46.6	± 7.5	27	53.3	± 9.6	49.5	± 10.2
Depression/dejection	*	27	51.2	± 11.6	47.4	± 9.7	27	52.9	± 9.7	49.0	± 9.5
Anger/hostility	*	27	48.9	± 8.2	45.5	± 6.6	27	49.7	± 11.2	46.5	± 10.7
Vigor/activity		27	55.2	± 9.4	54.8	± 9.4	27	49.2	± 9.8	49.8	± 10.0
Fatigue/inertia	*	27	47.5	± 9.3	46.6	± 7.1	27	53.2	± 11.0	48.5	± 11.9
Confusion/bewilderment		27	51.4	± 9.7	49.3	± 10.6	27	55.7	± 11.7	50.8	± 10.6
Total mood disturbance	†	26	189.9	± 35.9	178.3	± 35.4	27	215.5	± 49.7	194.5	± 51.0

n denotes the number of samples ; SD standard deviation.

* Normality was rejected by Shapiro-Wilk test, non parametric Mann-Whitney test for group difference.

† Homoscedasticity was rejected ; two-tailed Welch's t test was used for the group difference.

‡ denotes pre-intervention group differences determined to be significant by two-tailed independent t test with level of significance adjusted by Holm's method.

NK cell activity denotes natural killer cell activity at effector-to-target ratio of 20 : 1 ; T cell count, $CD3^+$ cell count ; B cell count, $CD20^+$ cell count ; CD4/CD8 ratio, ratio of $CD4^+$ cell count to $CD8^+$ cell count ; Naive T cell count, $CD45RA^+CD4^+$ cell count ; Memory T cell count, $CD45RA^-CD4^+$ cell count ; Naive/memory ratio, ratio of naive T cell count to memory T cell count ; NK cell count, $CD56^+CD16^+$cell count ; IFN-γ, interferon gamma ; IL, interleukin.

Mood state scores obtained using the Profile of Mood States questionnaire were transformed into T scores using the age distribution for healthy Japanese. Total mood disturbance represents a linear combination of the individual POMS scores inverting Vigor/activity.

Table 4. Mood States within and between groups (logarithmic scale).

Mood state	Change in older group n mean ± SD (p)	Change in younger group n mean ± SD (p)	Group difference mean ± SE (p)
Tension/anxiety	26 −0.057 ± 0.089 (0.003)	27 −0.078 ± 0.118 (0.002)	+0.021 ± 0.029 (0.468)
Depression/dejection	27 −0.073 ± 0.083 (0.000)	27 −0.076 ± 0.074 (0.000)	+0.003 ± 0.021 (0.888)
Anger/hostility	27 −0.070 ± 0.107 (0.001)*	27 −0.066 ± 0.078 (0.000)*	−0.003 ± 0.026 (0.993)*
Vigor/activity	27 −0.009 ± 0.129 (0.964)*	27 +0.011 ± 0.109 (0.608)*	−0.019 ± 0.033 (0.979)*
Fatigue/inertia	27 −0.013 ± 0.126 (0.322)*	27 −0.099 ± 0.133 (0.001)	+0.085 ± 0.035 (0.042)*
Confusion/bewilderment	27 −0.044 ± 0.145 (0.131)	27 −0.091 ± 0.107 (0.000)	+0.047 ± 0.035 (0.181)
Total mood disturbance	26 −0.065 ± 0.083 (0.001)	27 −0.109 ± 0.091 (0.000)	+0.044 ± 0.024 (0.074)

Change = $\log_e$[(post-intervention value)/(pre-intervention value)]. Group difference = mean change in the older minus mean change in younger. n denotes the total number of samples ; SD, standard deviation ; SE, standard error ; p, observed significance level of the test.
Two-tailed paired t-test was used for change ; two-tailed independent t-test for group difference.
Underlined values are significant according to the level of significance adjusted by Holm's method.
* Normality was rejected by Shapiro-Wilk test, non parametric Wilcoxon signed-ranks test was used for change, Mann-Whitney test for group difference.

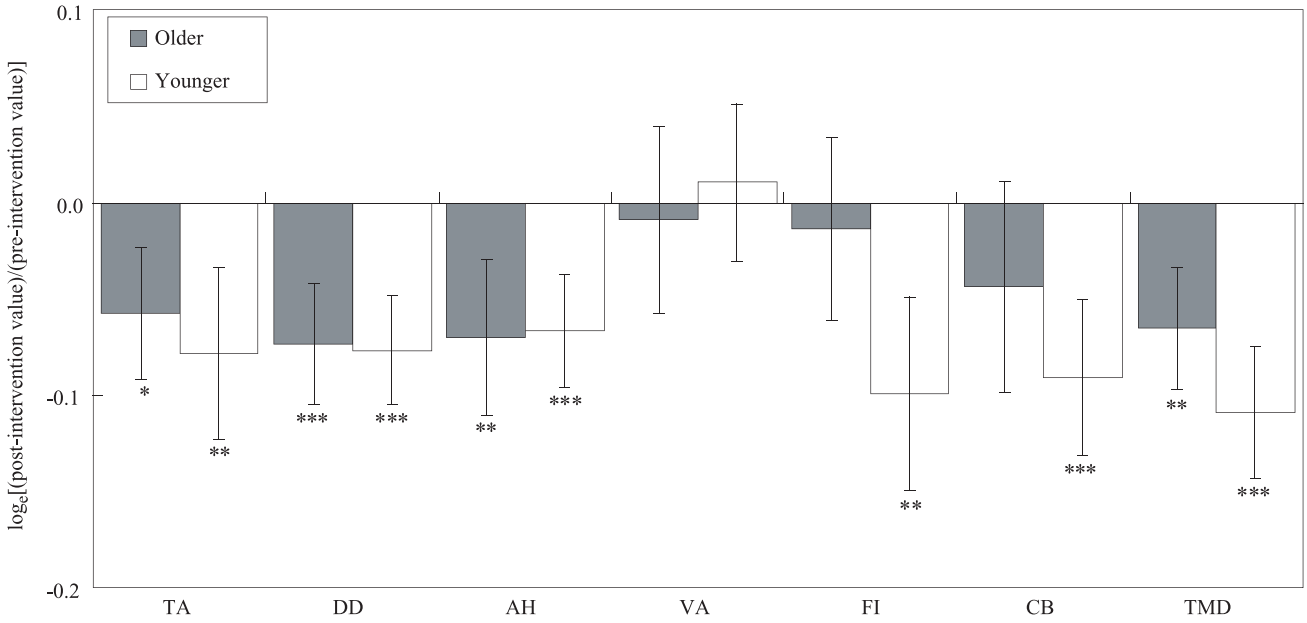

Fig. 1 : Comparison of the effect of RMM on mood states of the older and younger group. Mean normalized change ($\log_e$[(post-intervention value)/(pre-intervention value)]) is shown with 95% CI of mean indicated by error bars. Bars running in the positive direction indicate an average increase in the post-intervention value.
TA indicates tension/anxiety in POMS score ; DD, depression/dejection ; AH, anger/hostility ; VA, vigor/activity ; FI, fatigue/inertia ; CB, confusion/bewilderment ; TMD, total mood disturbance. *, significant after adjustment of the level of significance 0.05 by Holm's method ; **, after adjustment of 0.01 ; ***, after adjustment of 0.001.

Table 5. Biological parameters within and between groups (logarithmic scale).

	Change in older group			Change in younger group			Group difference		
Parameter	n	mean	± SD (p)	n	mean	± SD (p)	mean	± SE	(p)
Adrenaline	27	−0.068	± 0.266 (0.193)	27	+0.069	± 0.289 (0.225)	−0.137	± 0.075	(0.074)
Noradrenaline	27	+0.168	± 0.141 (0.000)*	27	+0.107	± 0.189 (0.007)	+0.062	± 0.045	(0.186)*
Dopamine	27	+0.040	± 0.248 (0.414)	27	+0.080	± 0.664 (0.939)*	−0.040	± 0.136	(0.478)*
NK cell activity	27	+0.058	± 0.164 (0.079)	27	−0.035	± 0.186 (0.335)	+0.093	± 0.048	(0.057)
White blood cells	27	+0.057	± 0.101 (0.007)	27	+0.020	± 0.118 (0.384)	+0.037	± 0.030	(0.219)
Neutrophils	27	+0.038	± 0.148 (0.203)*	27	+0.028	± 0.149 (0.564)*	+0.010	± 0.040	(0.736)*
Lymphocytes	27	+0.085	± 0.121 (0.001)	27	+0.020	± 0.135 (0.440)	+0.065	± 0.035	(0.048)*
T cells	27	+0.084	± 0.115 (0.001)	27	+0.036	± 0.137 (0.182)	+0.048	± 0.034	(0.171)
B cells	27	+0.152	± 0.268 (0.007)	27	+0.071	± 0.304 (0.237)	+0.081	± 0.078	(0.301)
$CD4^+$ T cells	26	+0.086	± 0.118 (0.001)	27	+0.019	± 0.163 (0.545)	+0.067	± 0.039	(0.094)
$CD8^+$ T cells	26	+0.053	± 0.139 (0.065)	27	+0.042	± 0.145 (0.149)	+0.011	± 0.039	(0.776)
CD4/CD8 ratio	26	+0.033	± 0.132 (0.219)	27	−0.022	± 0.105 (0.291)	+0.054	± 0.033	(0.102)
Naive T cells	26	+0.098	± 0.167 (0.006)	27	−0.019	± 0.241 (0.690)	+0.117	± 0.057	(0.047)
Memory T cells	26	+0.080	± 0.116 (0.002)	27	+0.053	± 0.157 (0.094)	+0.028	± 0.038	(0.469)
Naive/memory ratio	26	+0.012	± 0.122 (0.624)	27	−0.069	± 0.225 (0.055)*	+0.080	± 0.050	(0.031)*
NK cells	27	+0.026	± 0.273 (0.199)	27	−0.054	± 0.340 (0.239)*	+0.080	± 0.084	(0.102)*
IFN-γ production	26	+0.400	± 0.397 (0.000)	25	+0.145	± 0.661 (0.283)	+0.255	± 0.152	(0.100)
IL-2 production	26	+0.067	± 0.309 (0.620)*	25	+0.146	± 0.403 (0.201)*	−0.078	± 0.100	(0.598)*
IL-4 production	25	+0.061	± 0.594 (0.581)*	25	−0.066	± 0.413 (0.429)	+0.128	± 0.145	(0.421)*
IL-6 production	26	+0.552	± 0.378 (0.000)	25	−0.090	± 0.684 (0.517)	+0.642	± 0.156	(0.000)†
IL-10 production	26	−0.050	± 0.351 (0.471)	25	−0.050	± 0.455 (0.588)	−0.001	± 0.113	(0.996)

Change = $\log_e$[(post-intervention value)/(pre-intervention value)]. Group difference = mean change in older minus mean change in younger. n denotes the number of samples ; SD, standard deviation ; SE, standard error ; p, observed significance level of the test. Two-tailed paired t-test was used for change ; two independent t-test for group difference. Underlined values are significant according to the level of significance adjusted by Holm's method.

* Normality was rejected by Shapiro-Wilk test ; non-parametric Wilcoxon signed-ranks test was used for change ; Mann-Whitney test for the group difference.

† Homoscedasticity was rejected ; two-tailed Welch's t test was used for the group difference.

NK cell activity denotes natural killer cell activity at effector-to-target ratio of 20 : 1 ; T cells, $CD3^+$ cell count ; B cells, $CD20^+$ cell count ; CD4/CD8 ratio, ratio of $CD4^+$ cell count to $CD8^+$ cell count ; Naive T cells, $CD45RA^+CD4^+$ cell count ; Memory T cells, $CD45RA\text{-}CD4^+$ cell count ; Naive/memory ratio, ratio of naive T cell count to memory T cell count ; NK cells, $CD56^+CD16^+$cell count ; IFN-γ, interferon gamma ; IL, interleukin.

No significant difference was confirmed between sexes in both groups for the peripheral blood values.

Correlation of pre- and post-intervention parameters

A simple linear regression analysis was performed for production of IL-6 and IFN-γ that demonstrated significant changes in the older group (Fig. 3). In both groups, a positive correlation was found between IL-6 and IFN-γ (older group, $R^2 = 0.642$, $p < 0.001$; younger group, $R^2 = 0.271$, $p = 0.011$).

Exercise expenditure

Fig. 4 demonstrates total energy expenditure was less than 205 kilocalories per hour, or 3.41 kcal/min, for all participants, suggesting that the energy required for RMM participation.[14]

Discussion

The results of this study indicate that RMM affected the immunological responses of healthy older Japanese participants in a direction opposite to that expected as a result of chronic stress. The effects of RMM was, however, not significantly different between the younger group and the older group for all except one immunological parameter. In the older group,

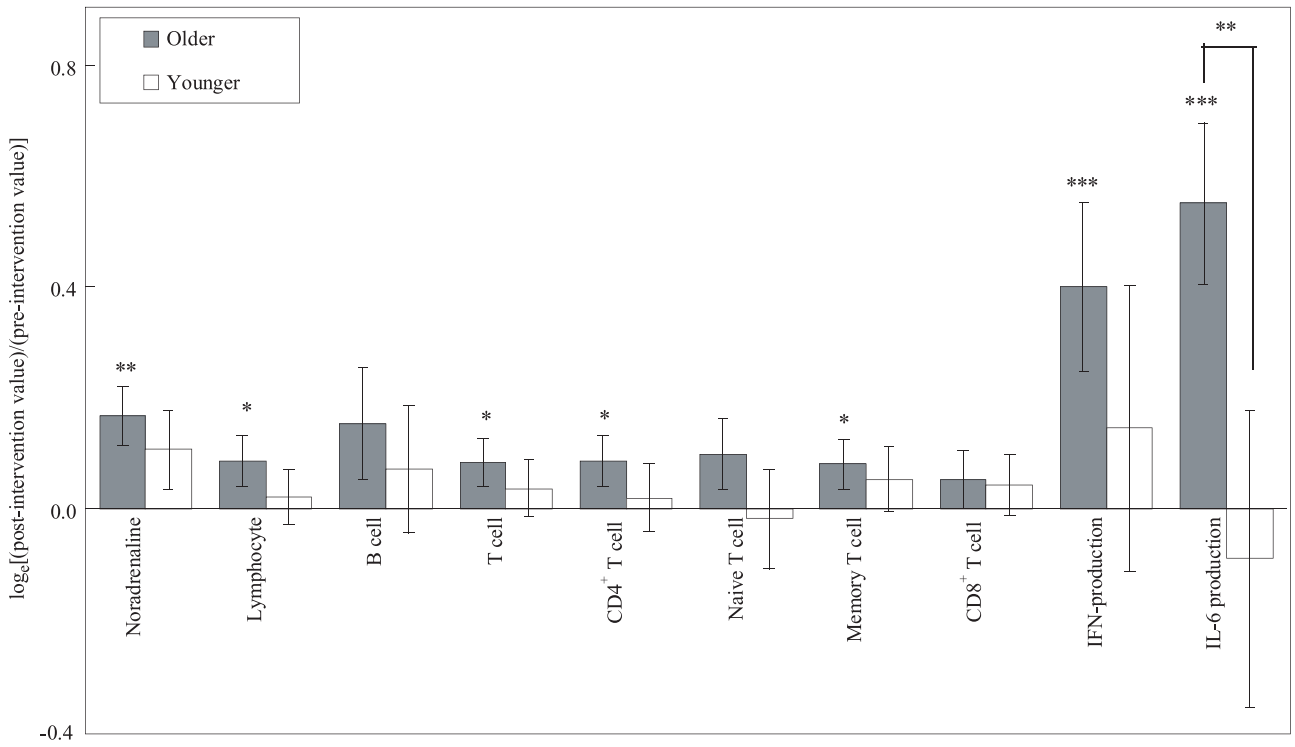

Fig. 2 : Comparison of the effect of RMM on biological parameters of the older and younger group. Mean normalized change ($\log_e$[(post-intervention value) / (pre-intervention value)]) is shown with 95% CI of mean indicated by error bars. Bars running in the positive direction indicate an average increase in the post-intervention value.
T cells denotes $CD3^+$ cell count ; B cells, $CD20^+$ cell count ; $CD4^+$ T cells, $CD4^+$ cell count ; Naive T cells, $CD45RA^+CD4^+$ cell count ; Memory T cells, $CD45RA^-CD4^+$ cell count ; $CD8^+$ T cells, $CD8^+$ cell count ; IFN-γ, interferon-gamma ; IL, interleukin. *, significant after adjustment of the level of significance 0.05 by Holm's method ; **, after adjustment of 0.01 ; ***, after adjustment of 0.001.

T cell counts, $CD4^+$ T cells, and memory T cells increased significantly after RMM. Furthermore, in the older group, levels of IFN-γ and IL-6 production also increased significantly, suggesting a functional improvement after RMM. The increase in Th1 cytokine IFN-γ, however, was not accompanied by changes in Th2 cytokine IL-4 and IL-10 levels, suggesting a shift to a Th1-dominant status in the older group, a shift opposite to that expected with stress. In the younger group, immunological changes were not significant. The only statistical group difference was in IL-6 production. Significant mood state improvements were noted in both the older and younger groups, but were not different between the two groups.

Immediately after the group drumming intervention, the number of lymphocytes in the older group increased statistically (Fig. 2). A significant increase in the number of T cells, a main component of lymphocytes, was also confirmed in the older group. Furthermore, significant increases were demonstrated in the number of $CD4^+$ T cells (a subset of T cells) and also in the number of memory T cells (a subset of $CD4^+$ T cells). In the younger group, no such significant immunological modulation was observed.

Of importance is the finding that the number of cells associated with stress and aging tended to increase in the older group after a one-hour RMM session. Reductions in the number of peripheral blood T cells and $CD4^+$ T cells have been associated with chronic stress [7,8,9] while decreases in the number of lymphocytes and T cells have been associated with aging.[1,10,11] It should be noted, however, that since cell counts were based on blood samples taken immediately after intervention, the increases in these cell counts in the present study are not likely to be due to a production of new immunological cells. They are more likely to be a result of changes in immunological cell distribution, i.e. lymphocytes in tissues flowing into peripheral blood. The persistence of these changes should be determined to assess the extent to which the intervention may have beneficial impact.

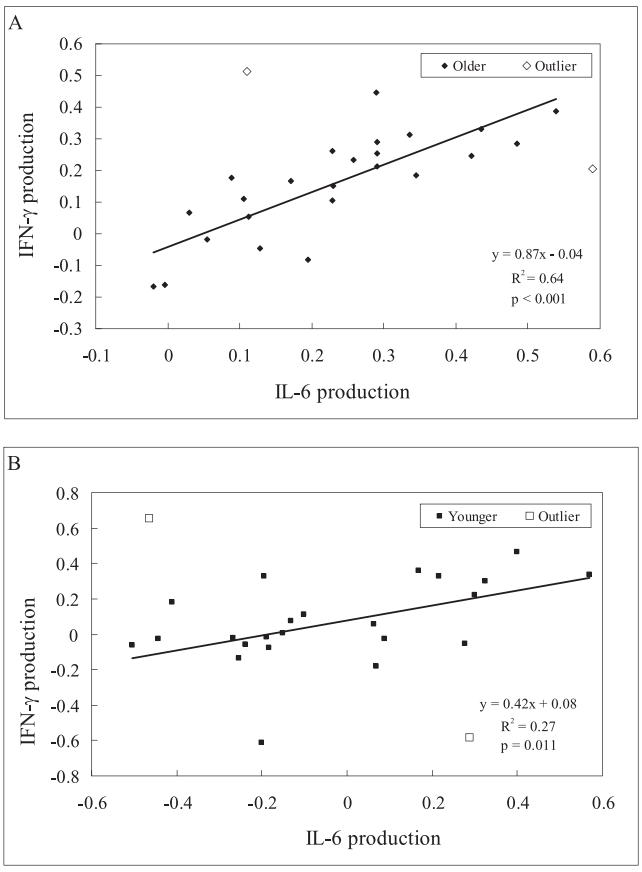

Fig. 3: Scatterplots of normalized changes ($\log_e$ [(post-intervention value) / (pre-intervention value)]) in IL-6 production versus IFN-γ production with linear regression analysis of data from the older and younger group. Open symbol denotes an outlier excluded from the analysis. A shows the older group. B shows the younger group. IFN-γ denotes interferon-gamma ; IL, interleukin.

Exposure to stress is thought to tip the balance between Th1 and Th2 cytokines to a Th2-dominant status,[20] resulting in a suppression of IFN-γ and IL-2 production and stimulation of IL-4 and IL-10 production.[21,22,23,24] The increase in Th1 cytokine IFN-γ and unchanged Th2 cytokine IL-4 and IL-10 levels in the older group suggests a shift to a Th1-dominant status, a shift opposite to that expected with stress. Also noted in the older group was an increased level of IL-6, a cytokine reported to stimulate production of IL-4.[25] The level of IL-4 production, however, was not increased significantly in the older group, suggesting it was not affected by the increase in IL-6. This leads us to speculate that the Th1/Th2 balance was unaffected by the increased level of IL-6 production. Therefore, the implications of cytokine production and Th1/Th2 balance remain unclear and warrant future exploration.

The overall improvement in mood subjectively reported by all participants supports the observation that RMM modulates the immunological profiles of both groups. Significant improvement was confirmed in five of the six mood states in the younger, and three in the older group together with a decrease in the total mood disturbance in both groups. No significant group difference was found, indicating that the RMM impact did not differ between the two age groups.

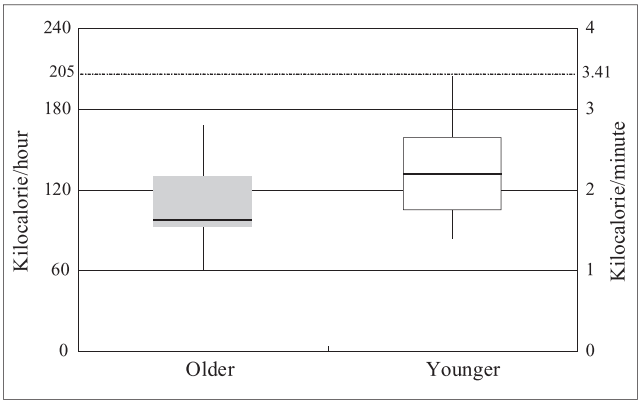

Fig. 4 : Box plot showing distribution of participants' kilocalorie consumption per hour for the two groups during the one-hour RMM session.

Noradrenaline level after RMM was increased only in the older group. Since blood noradrenaline increases are associated with emotional upsurge, especially involving active activities or excitement,[26] the significant increases confirmed in both groups may be attributed to temporary excitement associated with the intervention. It is impossible, however, to draw specific mechanistic conclusions since factors other than emotion such as diurnal variations in noradrenaline levels may have played a role in our findings.

NK cell activity results were also inconclusive in determining the effect of RMM for the two groups. Our findings were similar to those of Wachi et al.[6] in that NK cell activity in both the older and younger groups tended to increase when initially low and decrease when high, indicating alleviation of stress. Regression analyses of pre-intervention values and the difference between pre- and post-intervention values disclosed no significant difference (Fig. 5).

Significant pre-intervention group differences in immunological profiles (Table 3) confirm immunological differences between age groups noted by Hirokawa et al.[1,11] Hirokawa et al. reported an age-related increase in $CD4^+$ T cell counts, memory T cell counts, and the

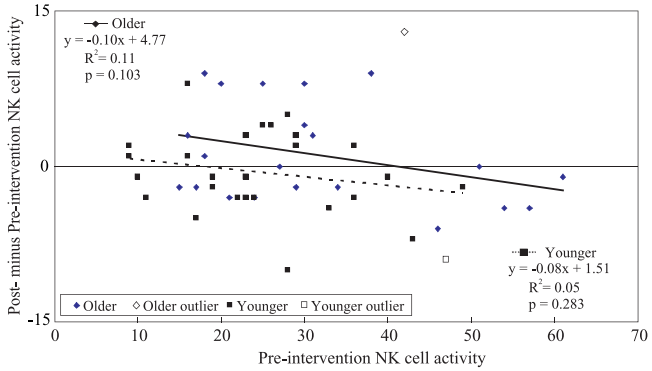

Fig. 5 : Scatterplots of Natural Killer (NK) cell activity at effector-to-target ratio of 20 : 1 in original pre-intervention value versus original change (original post-intervention value minus original pre-intervention value) with linear regression analysis of data from the older and younger group. Percent specific lysis (% lysis) is plotted. Points over the line y = 0 indicated an increase of NK cell activity after intervention. Outliers (open symbols) had Cook's distance larger than 4/n, and were excluded from the analysis. Homogeneity of pre-change partial regression coefficient was not rejected with p = 0.103 in the older group and p = 0.283 in the younger group. Sample size after excluding outliers was 26 in the older and 26 in the younger group.

ratio of $CD4^+$ T cells to $CD8^+$ T cells. They also noted an age-related decrease in the number of B cells, $CD8^+$ T cells, T cells, and naive T cells.[11] In the present study, the pre-intervention group differences for all these parameters reflected the reported differences except for T cell counts and naive T cell counts, both of which were greater in older people.

No statistically significant difference was noted between men and women in the older group for both immunological parameters and mood states.

The amount of energy required for participation in this RMM session was less than that required for walking.[14] Furthermore, participants were not required to perform rigorous physical activity and were seated for most of the one-hour session. The low level of energy and physical activity required suggest that this intervention is a suitable endeavor for the older generation or people who are unable to engage in strenuous activity.

Obvious limitations of this study include sample size, a disproportionately large number of women in the younger group, and frequency of intervention. A long-term evaluation of this RMM program on a larger subject sample with control groups could provide more definitive results. Studies involving two or more groups of older adults might also provide further clarification of age-related biological and psychosocial responses.

Conclusion

This RMM group drumming intervention improved mood and modulated certain immunological responses of healthy older Japanese participants in a direction opposite to that expected with chronic stress. The changes were not different between groups except for one immunological parameter. Specifically, in the older group, we detected significant increases in the numbers of lymphocytes, T cells, and T cell subsets ($CD4^+$ T cell and memory T cells) , indicating immunological changes in a direction opposite to those associated with aging and chronic stress. Furthermore, we suspect that significant increases in IFN-γ and unchanged IL-4 levels shifted the Th1/Th2 balance thereby eliciting changes in a direction opposite to those expected in association with chronic stress in the older group. Immunological modulation was also confirmed in the younger group, but none of the changes were significant. Significant improvement was confirmed in five of the six mood states in the younger, and three in the older group together with a decrease in the total mood disturbance in both groups. The changes in mood states were not statistically significant between the two groups. The lack of group difference in both immunological responses and mood states suggests that this RMM protocol is not more effective for the older group than for the younger. However, the modulation of both the immunological profile and mood states in the older group and the minimal energy required for participation establishes this protocol as a suitable activity for older adults. Ultimately, our composite findings suggest this RMM protocol is a potentially valuable wellness activity for older Japanese men and women.

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participants.

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