16) Thase ME, Rush AJ, Howland RH *et al*：Double-blind switch study of imipramine or sertraline treatment of antidepressant-resistant chronic depression. *Arch Gen Psychiatry* **59**：233-239, 2002

17) Nolen WA, van de Putte JJ, Dijken WA *et al*：Treatment strategy in depression. I. Non-tricyclic and selective reuptake inhibitors in resistant depression：a double-blind partial cross-over study on the effects of oxaprotiline and fluvoxamine. *Acta Psychiatr Scand* **78**：668-675, 1988

18) Delgado PL, Price LH, Charney DS *et al*：Efficacy of fluvoxamine in treatment-refractory depression. *J Affect Disord* **15**：55-60, 1988

19) Poirier MF, Boyer P：Venlafaxine and paroxetine in treatment-resistant depression. Double-blind, randomised comparison. *Br J Psychiatry* **175**：12-16, 1999

20) 井上猛，北市雄士，小山司：治療抵抗性うつ病の治療戦略とその作用機序．日本精神神経学雑誌 **106**：1016-1023，2004

21) Rush AJ, Trivedi M, Fava M：Depression, IV：STAR*D treatment trial for depression. *Am J Psychiatry* **160**：237, 2003

22) Trivedi MH, Rush AJ, Wisniewski SR *et al*：Evaluation of outcomes with citalopram for depression using measurement-based care in STAR*D：implications for clinical practice. *Am J Psychiatry* **163**：28-40, 2006

23) Rush AJ, Trivedi MH, Wisniewski SR *et al*：Bupropion-SR, sertraline, or venlafaxine-XR after failure of SSRIs for depression. *N Engl J Med* **354**：1231-1242, 2006

24) Papakostas GI, Petersen T, Worthington JJ *et al*：A pilot, open study of sertraline in outpatients with treatment-resistant depression (TRD) or with a history of TRD who responded but later relapsed. *Int Clin Psychopharmacol* **18**：293-296, 2003

25) Gagiano CA, Muller PG, Fourie J *et al*：The therapeutic efficacy of paroxetine：(a) an open study in patients with major depression not responding to antidepressants；(b) a double-blind comparison with amitriptyline in depressed outpatients. *Acta Psychiatr Scand* **80** (suppl 350)：S130-S131, 1989

Eur Arch Psychiatry Clin Neurosci (2007) 257:149–152 DOI 10.1007/s00406-006-0698-7

ORIGINAL PAPER

Seiji Hama · Hidehisa Yamashita · Masaya Shigenobu · Atsuko Watanabe · Kaoru Kurisu
Shigeto Yamawaki · Tamotsu Kitaoka

Post-stroke affective or apathetic depression and lesion location: left frontal lobe and bilateral basal ganglia

Received: 28 March 2006 / Accepted: 28 August 2006 / Published online: 25 November 2006

■ **Abstract** This study was designed to examine the correlation between damage to the basal ganglia or frontal lobe and depression status (both affective and apathetic dimensions) in 243 stroke patients. We assessed the affective dimension in post-stroke depression (PSD) using the Zung Self-rating Depression Scale (SDS) and the apathetic dimension in PSD using the apathy scale (AS). We classified basal ganglia or frontal lobe damage into four groups: no damage, damage to the left side only, damage to the right side only, and damage to both sides. Affective and/or apathetic PSD was found in 126 patients (51.9%). The severity of affective depression (SDS score) was associated with left frontal lobe (but not basal ganglia) damage, and that of apathetic depression (AS score) was related to damage to the bilateral basal ganglia (but not to the frontal lobe). The anatomical correlates of PSD differ depending on the PSD dimension (affective or apathetic) and may explain interstudy differences regarding the association between lesion location and type of PSD.

■ **Key words** basal ganglia · frontal lobe · stroke · apathy · Zung Self-rating Depression Scale

S. Hama, M.D. · M. Shigenobu, M.D. · A. Watanabe M.D.
T. Kitaoka, M.D.
Department of Rehabilitation
Nishi-Hiroshima Rehabilitation Hospital
Hiroshima, Japan

H. Yamashita, M.D. · S. Yamawaki, M.D.
Department of Psychiatry and Neuroscience
Graduate School of Biomedical Science
Hiroshima University
Hiroshima, Japan

K. Kurisu, M.D.
Department of Neurosurgery
Graduate School of Biomedical Science
Hiroshima University
Hiroshima, Japan

S. Hama, M.D. (✉)
Nishi-Hiroshima Rehabilitation Hospital
Miyake 265
Saeki-ku
Hiroshima 731-5143, Japan
Tel.: +81-82/921-3230
Fax: +81-82/921-3237
E-Mail: wel@welnet.jp

Introduction

Depression is a common neuropsychiatric consequence of stroke and has been reported to negatively affect functional and cognitive recovery (Alexopoulos et al. 1997; Biringer et al. 2005). Some studies, including those on stroke patients, have demonstrated morphological changes in major depression with respect to the hippocampus, basal ganglia and frontal lobe (Alexopoulos et al. 1997; Bielau et al. 2005; Frodl et al. 2004; Sheline et al. 1996). Therefore, the neuroanatomical model of mood regulation was developed from the observation that lesions in some cortical/subcortical regions resulted in depression (Soares and Mann 1997).

"Depressed mood" is a very sensitive symptom in the diagnosis of depression (affective PSD) using Self-rating Depression (SDS), which is a widely used self-report questionnaire used to measure depression (Kitamura et al. 2004). On the other hand, "loss of interest" is a less sensitive symptom than "depressive mood" using SDS and is thought to be a component of apathy, which is often observed after stroke and is defined as reduced motivation and lack of initiative and exploration (Starkstein et al. 1993; Yamagata et al. 2004).

In the present study, we examined the affective and apathetic dimensions of post-stroke depression (PSD) separately, and evaluated their correlation with basal ganglia or frontal lobe damage in stroke patients.

Subjects and methods

Patients

The approval of our institutional ethics committee was obtained for this prospective study. Informed consent was obtained from all patients. Patients included in this study were selected from a consecutive series of 408 patients with hemorrhagic or occlusive stroke, who were diagnosed using computed tomography (CT), and who were admitted to the Nishi-Hiroshima Rehabilitation Hospital less than 3 months after suffering their stroke. Exclusion criteria included (1) history of major psychiatric illness (seven patients); (2) medical illness (four patients) or speech impediment (117 patients) that may affect cognitive function and ability to provide consent; (3) subarachnoid hemorrhage (25 patients); (4) physical disability which precludes cognitive testing (12 patients). The remaining 243 patients were included as subjects in this study.

CT findings

The study had a naturalistic design using 243 CT scans. As such a high sample size would not have been possible with MR, the CT technique used is adequate. CT scanning was performed on all patients at admission (with a follow-up CT scan every 1-3 months after admission). Damage to the basal ganglia or frontal lobe (including lacunar infarcts) was defined as a sharply demarcated hypodense lesion with a diameter >5 mm on CT. We classified patients into four groups according to the degree of basal ganglia or frontal lobe damage: no damage, damage to the left side only, damage to the right side only, and damage to the bilateral basal ganglia or frontal lobe. The measurement of the volume of CT-defined LDA was calculated according to the formula $0.5 \times A \times B \times C$; where A and B represent the largest perpendicular diameters and C is the thickness (Montaner et al. 2001).

Psychological assessment

We used the Japanese version of the SDS to examine the subjective severity of affective depression (Yamaguchi et al. 1992) and used a Japanese version of the apathy scale (AS) to quantify the apathetic state (Yamagata et al. 2004). We classified the patients into two groups according to their test scores: a non-depressed group (SDS score < 45 points) and a depressed group (SDS score ≥ 45 points), and a non-apathetic group (apathy score < 16 points) and an apathetic group (apathy score ≥ 16 points). The cut-off point was determined on the basis of a previous report on Japanese stroke patients (Yamaguchi et al. 1992).

Statistical analyses

Different degrees of basal ganglia or frontal lobe damage (none, left only, right only and bilateral) were compared with SDS or AS scores by one-way analysis of variance (ANOVA) followed by a post-hoc Fisher protected least significant difference test (Fisher PLSD test). Values were considered to be significant at $P < 0.05$. The Stat View 5.0 (SAS Institute, Inc., Cary, NC) statistical package was used for all analyses.

Results

Baseline structures and the frequency of PSD in all patients

The subjects consisted of 162 males and 81 females (age: 65.2 ± 11.3, past history of stroke: 27 cases (11.1%), time interval between onset and admission: range 7–90 days, mean 40.7 ± 19.6 days). SDS and AS scores over the cut-off limits were observed in 79 (32.5%) and 98 (40.3%) patients, respectively. Of these, 50 patients (20.6%) showed elevation of both SDS and AS, therefore 126 patients (51.9%) were found to have affective and/or apathetic PSD.

The effects of lesion location on affective and/or apathetic PSD

Computed tomography densities of left side and cortical lesions (especially middle cerebral arterial territory damage; e.g., temporal lobe) were found to be greater in speech impaired patients (excluded from this study) than in other patients.

The severity of affective or apathetic PSD was related to CT-defined lesion volume (Mann–Whitney U-test, $P < 0.02$), consistent with a previous report (Nys et al. 2005). But no association could be demonstrated between severity of PSD and lesion location involving supra-or infra-tentorial LDA (Mann–Whitney U-test, $P > 0.2$), so further examinations were made of supratentorial stroke lesions in the frontal lobe and basal ganglia.

The SDS score increased significantly from none, right side only, left side only, to bilateral frontal lobe damage ($P = 0.0450$, ANOVA test) (Fig. 1). Post-hoc testing (Fisher's test) showed a difference between no damage and damage to the left side only ($P = 0.0237$). However, we did not find significant differences in AS scores in relation to frontal lobe damage.

The AS score increased significantly from none, left side only, right side only, to bilateral BG damage ($P = 0.0061$, ANOVA test) (Fig. 1). Post-hoc testing (Fisher's test) showed a difference between no damage and bilateral BG damage ($P = 0.0024$), and between left side only and bilateral BG damage ($P = 0.0075$). However, we did not find significant differences in SDS scores in relation to BG damage.

Discussion

We found that the severity of affective depression (SDS score) was associated with left frontal lobe damage, but not damage to the basal ganglia. Apathetic depression (AS score) was not related to frontal lobe damage but was related to damage to the basal ganglia in both the right and left hemispheres.

Although there is conflicting evidence as to whether the risk of depression after stroke is influenced by the location of the brain lesion, several explanations can be proposed for our findings. Soares and Mann (1997) suggested that functional abnormalities in frontal, subcortical, and limbic structures appear to be part of the pathophysiology of depression. Robinson et al. (1984) and Starkstein et al. (1987) exam-

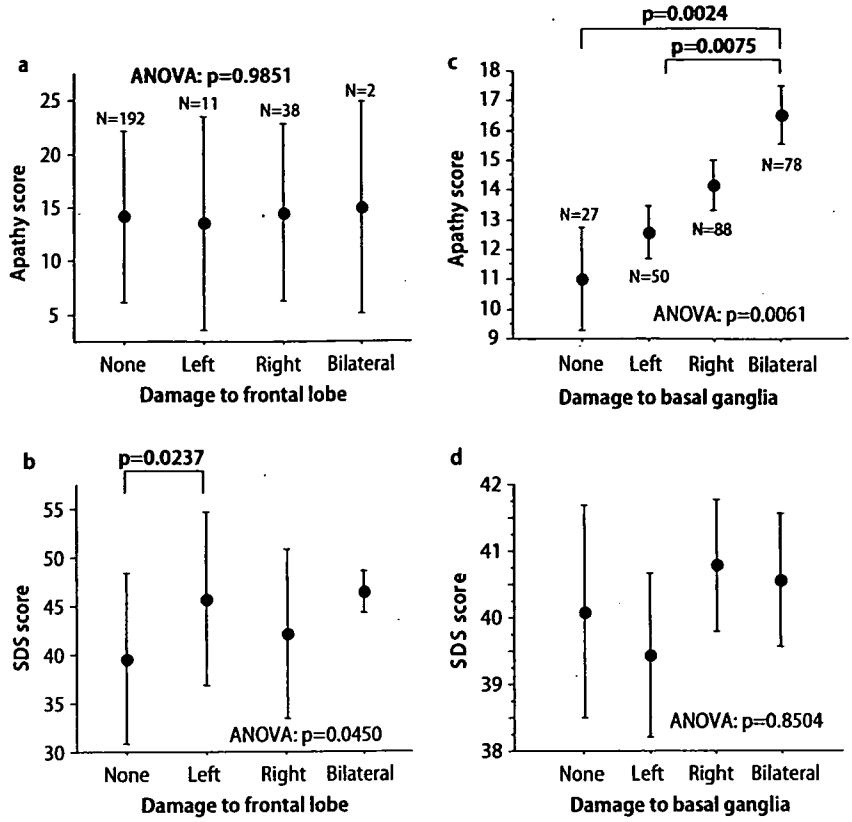


Fig. 1 Differences in apathy score (**A, C**) and SDS score (**B, D**) between patients with no damage, left side only, right side only, and bilateral damage to the frontal lobe (**A, B**) or basal ganglia (**C, D**). The midpoint, top and bottom of each vertical line represent the mean, upper, and lower 95% CI values, respectively. ANOVA of the four frontal lobe or basal ganglia damage subgroups shows significant results for SDS (**B**) or AS (**C**). The Fisher PLSD test also indicates that these parameters can distinguish between some of these SDS or AS subgroups, with the *P*-values given

ined depression in stroke patients using SDS, and their findings suggested a strong correlation between the severity of depression and proximity of the lesion to the frontal pole. Other studies also noted a significant association between strokes affecting the frontal lobe (or anterior part) of the left hemisphere and PSD (Alexopoulos et al. 1997). These reports agree with our findings that "depressed mood (affective PSD)" is associated with the left frontal lobe.

Starkstein et al. (1993) suggested that apathy was significantly associated with lesions involving the posterior limb of the internal capsule. Later, Yamagata et al. (2004) examined the relationship between apathy after subcortical stroke and neural orienting response to novel events using an event-related evoked potential technique, suggesting that apathy after subcortical (include basal ganglia) stroke is intimately linked to dysfunction of the frontal-subcortical system. These findings agree with our data, indicating that damage to basal ganglia leads to dysfunction of the frontal-subcortical system, resulting in apathy after stroke.

Several methodological limitations of this study should be acknowledged. First, patients with severe comprehension deficits and/or severe speech impediments, who had different lesion patterns compared to the other patients, were excluded from the study. Therefore, the results of this study may be biassed. Second, patients enrolled in this study only underwent CT scanning (not MRI). Thus, the presence of lesions that could not be visualized by CT may have influenced our findings. Third, we used a self-report questionnaire to measure the level of depression. Thus, the absence of objective assessment may have influenced our findings.

In summary, this study found that after a stroke there are two separate core symptoms ("depressed mood" or "loss of interest") with different underlying neuroanatomical mechanisms. Therefore, in order to help patients gain independence, future studies should examine whether these different lesion correlates of affective or apathetic PSD, separately or together, may also be reflected in different patterns of treatment response.

■ **Acknowledgment** This study was supported by the Ministry of Health, Labour and Welfare, Japan.

References

1. Alexopoulos GS, Meyers BS, Young RC, Campbell S, Silbersweig D, Charlson M (1997) 'Vascular depression' hypothesis. Arch Gen Psychiatry 54:915–922
2. Bielau H, Trübner K, Krell D, Agelink MW, Bernstein HG, Stauch R, Mawrin C, Danos P, Gerhard L, Bogerts B, Baumann B (2005) Volume deficits of subcortical nuclei in mood disorders: A postmortem study. Eur Arch Psychiatry Clin Neurosci 255:401–412

3. Biringer E, Lundervold A, Stordal K, Mykletun A, Egeland J, Bottlender R, Lund A (2005) Executive function improvement upon remission of recurrent unipolar depression. Eur Arch Psychiatry Clin Neurosci 255:373–380
4. Frodl T, Meisenzahl EM, Zetzsche T, Hohne T, Banac S, Schorr C, Jager M, Leinsinger G, Bottlender R, Reiser M, Moller HJ (2004) Hippocampal and amygdala changes in patients with major depressive disorder and healthy controls during a 1-year follow-up. J Clin Psychiatry 65:492–499
5. Kitamura T, Hirano H, Chen Z, Hirata M (2004) Factor structure of the Zung Self-rating Depression Scale in first-year university students in Japan. Psychiatry Res 128:281–287
6. Montaner J, Alvarez-Sabín J, Molina C, Anglés A, Abilleira S, Arenillas J, González MA, Monasterio J (2001) Matrix metalloproteinase expression after human cardioembolic stroke: temporal profile and relation to neurological impairment. Stroke 32:1759–1766
7. Nys GM, van Zandvoort MJ, van der Worp HB, de Haan EH, de Kort PL, Kappelle LJ (2005) Early depressive symptoms after stroke: neuropsychological correlates and lesion characteristics. J Neurol Sci 228:27–33
8. Robinson RG, Kubos KL, Starr LB, Rao K, Price TR (1984) Mood disorders in stroke patients. Importance of location of lesion. Brain 107:81–93
9. Sheline YI, Wang PW, Gado MH, Csernansky JG, Vannier MW (1996) Hippocampal atrophy in recurrent major depression. Proc Natl Acad Sci USA 93:3908–3913
10. Soares JC, Mann JJ (1997) The anatomy of mood disorders-review of structural neuroimaging studies. Biol Psychiatry 41:86–106
11. Starkstein SE, Robinson RG, Price TR (1987) Comparison of cortical and subcortical lesions in the production of post-stroke mood disorders. Brain 110(Pt 4):1045–1059
12. Starkstein SE, Fedoroff JP, Price TR, Leiguarda R, Robinson RG (1993) Apathy following cerebrovascular lesions. Stroke 24:1625–1630
13. Yamagata S, Yamaguchi S, Kobayashi S (2004) Impaired novelty processing in apathy after subcortical stroke. Stroke 35:1935–1940
14. Yamaguchi S, Kobayashi S, Koide H, Tsunematsu T (1992) Longitudinal study of regional cerebral blood flow changes in depression after stroke. Stroke 23:1716–1722

Attenuated Anterior Cingulate Activation During a Verbal Fluency Task in Elderly Patients With a History of Multiple-Episode Depression

Hiroshi Takami, M.D., Yasumasa Okamoto, M.D., Ph.D., Hidehisa Yamashita, M.D., Ph.D., Go Okada, M.D., Ph.D., Shigeto Yamawaki, M.D., Ph.D.

Objective: *Previous studies have suggested that, in elderly patients, prior depression plays a role in the recurrence of depression. The aim of this study was to investigate cerebral brain function in recovered depressed elderly and investigate the relationship between this brain function and the number of depressive episodes.* **Methods:** *Twenty elderly depressive patients in recovery and 10 healthy volunteers were included in this study. The depressive patients were divided into those who had experienced a single depressive episode and those who had experienced multiple episodes. Functional magnetic resonance imaging was performed in each participant during a verbal fluency task. The data were analyzed using statistical parametric mapping.* **Results:** *Activation in the anterior cingulate cortex was significantly attenuated in patients who had experienced multiple depressive episodes, compared with the other two groups. There were no significant differences in areas of activation between patients with a single depressive episode and healthy volunteers.* **Conclusions:** *These findings suggest that attenuated activation in the anterior cingulate cortex may be associated with multiple episodes of depression in the elderly and with the vulnerability to cycling or recurrence.* (Am J Geriatr Psychiatry 2007; 15:594-603)

Key Words: Elderly depression, recurrence, cycling, anterior cingulate cortex, functional magnetic resonance imaging

In elderly patients with a history of depression, the risk of relapse is higher than in younger patients.[1-4] Evidence from several studies suggests that elderly patients with a history of multiple episodes of depression are at high risk of recurrence,[5,6] and that prior depression appears to be

Received August 6, 2006; revised September 22, 2006; accepted November 6, 2006. From the Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University (HT, YO, HY, SY) and Mihara Hospital (GO), Hiroshima, Japan. Send correspondence and reprint requests to Shigeto Yamawaki, MD, PhD, Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. e-mail: yamawaki@hiroshima-u.ac.jp.

an important risk factor for depression in the elderly.[7]

Treatment response and neuropsychological function also appear to be impaired in elderly patients who have experienced multiple depressive episodes compared with those who have experienced a single episode of depression.[8] Furthermore, multiple previous depressive episodes have been linked with neuropsychological impairment in the euthymic phase of depression.[9] These findings suggest that the presence of prior depressive episodes in elderly patients might affect their neuropsychological function and treatment response.

Previous functional neuroimaging studies in the depressed elderly[10–17] have suggested global and regional decreases in cerebral perfusion and glucose metabolism, particularly in the prefrontal and anterior cingulate cortex. However, results regarding the presence of this hypoperfusion and hypometabolism after remission and recovery from depression in the depressed elderly are inconsistent.

Although clinically the presence of a prior depressive episode appears to play a role in relapse and recurrence in elderly depression, the pathophysiological mechanisms involved are unclear. We hypothesized that there may be differences in the cerebral brain function of the depressed elderly that are related to the number of prior depressive episodes. However, to our knowledge, no previous functional neuroimaging study has been conducted to specifically examine this relationship. The aim of this study was to investigate the effect of the number of prior depressive episodes on cerebral brain function in recovered depressed elderly. Therefore, using functional magnetic resonance imaging (MRI) during a verbal fluency task which activates the prefrontal and anterior cingulate cortex,[18] we compared brain activity among patients with a single depressive episode, those with multiple episodes and healthy comparison subjects.

METHODS

Subjects

The study included 20 elderly patients in recovery from depression and 10 healthy volunteers. All subjects were Japanese, aged over 50 years and right-handed (according to the Edinburgh Handedness Inventory).[19] All depressed patients were outpatients at Hiroshima University Medical Hospital. They were received clinical interviews in a systematic manner by senior psychiatrists and diagnosed as having experienced one or more major depressive episodes according to *Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM–IV)* criteria.[20] Exclusion criteria included a history of psychosis, mania, or other axis I disorder; history of neurological illness; and serious or unstable current medical condition. All of them had no psychiatric comorbidity and no family history of major depressive disorder in the first and the second degree of relationship. The age at onset of the first depressive episode in these 20 patients was over 50 years. They were in recovery and were being treated with average dosage of antidepressants for maintenance therapy at the time of this study. All antidepressants dosages were converted to an equivalent imipramine dosage according to Inagaki's report.[21] Recovery was defined as maintaining a score of no more than seven on the 17-item Hamilton Rating Scale for Depression[22] for over 3 months. Patients were divided into two groups according to the number of prior depressive episodes: a single-episode group (group 1: 10 subjects with a single depressive episode) and a multiple-episode group (group 2: 10 subjects with multiple depressive episodes). Of the patients in group 2, eight had experienced two depressive episodes and two had experienced three episodes. The last episode-free period was investigated for all patients and the score on the Global Assessment of Functioning scale was ascertained at the time of this study. The comparison group included 10 healthy volunteers comparable in age and education with the patients recruited from the local community. All of them had no current or lifetime psychiatric illness. No study subjects suffered from dementia as assessed by the Mini-Mental State Examination.[23] Detailed patient characteristics are outlined in Table 1.

The study was conducted under a protocol approved by the Ethics Committee of Hiroshima University School of Medicine. All subjects submitted informed written consent prior to their participation.

Experimental Paradigm

We used a periodic design involving the presentation of a baseline condition for 30 seconds followed by

TABLE 1. Demographic Details of the Three Groups

	Comparison	Single-Episode Group	Multiple-Episode Group	Degrees of Freedom	p Value
Number	10	10	10		
Sex (M/F)[a]	4/6	5/5	3/7	2	0.659
Age[b]	67.6 ± 9.7	65.9 ± 7.8	62.5 ± 9.1	2, 27	0.437
Year of education[b]	11.8 ± 2.6	12.1 ± 3.7	12.9 ± 2.1	2, 27	0.680
Score on MMSE[b]	29.0 ± 1.0	29.0 ± 1.0	28.8 ± 1.5	2, 27	0.899
Age at onset of first episode[c]		62.8 ± 7.7	57.0 ± 7.2	18	0.099
Months after last episode[c]		24.8 ± 18.9	23.9 ± 24.1	18	0.927
Score on HRSD[c]		3.2 ± 2.3	3.1 ± 2.2	18	0.922
Score on GAF scale[c]		81.0 ± 9.4	81.0 ± 8.8	18	0.999
Converted dosage of antidepressants (mg)[c]		49.0 ± 49.6	59.5 ± 54.2	18	0.657
Classes of antidepressants					
SSRI or SNRI/TCA or others/none)		3	4		
TCA or others		4	4		
None		3	2		
Adjuvant psychotropic drugs					
BDZs		7	7		
Anticonvulsants		0	0		
Antipsychotics		0	0		

Notes: Results are means ± SD. All antidepressants dosages were converted to an equivalent imipramine dosage. MMSE: Mini-Mental State Examination; HRSD: Hamilton Rating Scale for Depression; GAF: Global Assessment of Functioning; VFT: Verbal Fluency Task; SSRI: selective serotonin reuptake inhibitor; SNRI: serotonin norepinephrine reuptake inhibitor; TCA: tricyclic antidepressant; BDZ: benzodiazepine.

[a]Chi-square test.

[b]Analysis of variance.

[c]Unpaired *t*-test.

an activation condition for 30 seconds. This cycle was repeated three times over the course of three minutes. During the activation condition, subjects were cued by visual presentation of one of three letters (the Japanese phonetic characters that are pronounced "sa," "ta," and "te") and asked to generate a word beginning with that letter and to internally articulate the word but not to use the same word repeatedly. One of the three letters was presented every three seconds. During the baseline condition, subjects were cued by visual presentation of the word *yasumi* (which means "rest") every three seconds and asked to internally articulate that word. Before image acquisition, all subjects performed the following performance test outside of the scanner: using the same design as above with three different letters ("ka," "na," and "to" instead of "sa," "ta," and "te"). On this test, subjects were instructed to articulate the words externally, but not internally, and the number of words generated was recorded.

Image Acquisition

Functional MRI (fMRI) was performed using a Magnetom Symphony Maestro Class (Siemens, Tokyo) at Kajikawa Hospital. A time-course series of 64 image volumes was acquired with T2*-weighted, gradient echo, echo planar imaging (EPI) sequences. Each image volume consisted of 38 slices; the slice thickness was 4 mm with no gap, and covered the entire cerebral and cerebellar cortices. The interval between two successive acquisitions of the same image (TR) was 3,000 msec, the echo time (TE) was 48 msec, and the flip angle was 90°. The field of view (FOV) was 256 mm, and the matrix size was 64×64, giving voxel dimensions of 4×4×4 mm. Scan acquisition was synchronized to the onset of the trial. After functional scanning, structural scans were acquired using a T1-weighted gradient echo pulse sequence (TR = 2,050 msec; TE = 3.93 msec; flip angle = 15°; FOV = 256 mm; voxel dimensions of 1×1×1 mm), which facilitated localization.

Data Analysis

For statistical analysis, data were subjected to analysis of variance (ANOVA), chi-square test, and unpaired *t*-test for comparison of the demographic de-

tails of each group. Significance was defined as $p < 0.05$.

For image processing and statistical analyses we used the statistical parametric mapping (SPM99) software (Wellcome Department of Cognitive Neurology, London) implemented in Matlab (Mathworks Inc., Sherborn, MA). The first image volume was discarded because magnetization was unsteady, and the last three image volumes were discarded as they were taken during the posttask period. The remaining 60 image volumes were used for statistical analysis. Images were corrected for motion and realigned with the first scan of the session as a reference. T1 anatomical images were registered to the first functional image in each subject and aligned to a standard stereotaxic space, using Montreal Neurological Institute (MNI) T1 template in SPM99. The calculated nonlinear transformation was applied to all functional images for spatial normalization. Finally, the functional images were smoothed with a 10-mm full-width, half-maximum Gaussian filter.

Using group analysis according to a random effect model that allowed inference to the general population,[24] we identified brain regions that showed significant responses during word generation compared with word repetition. The group analysis consisted of two levels. In the first level, the signal time course of each subject was modeled with a delayed box-car function convolved with a hemodynamic response function in the context of a general linear model. One contrast image per subject was created by contrasting word generation with word repetition. In the second step, these images were entered into a one-sample *t*-test and then a two-sample *t*-test. The resulting foci were then characterized in terms of spatial extent (k) and peak height (u). The significance of each region was estimated using distribution approximations from the theory of Gaussian fields. This characterization was in terms of the probability that a region of the observed number of voxels (or greater) could have exceeded the determined threshold by chance (extent threshold), or that the peak height found (or higher) could have occurred by chance (height threshold) over the entire volume analyzed. Activations were reported if they exceeded $p < 0.001$ (uncorrected) on the single voxel level and $p < 0.05$ (corrected) on the cluster level. Significance on the cluster level was calculated considering peak activation and extent of the cluster.

The xyz coordinates provided by SPM, which are in the MNI brain space, were converted to xyz coordinates in Talairach and Tournoux's (TT) brain space[25] using the following formulas: $TT - x = MNI - x \times 0.88 - 0.8$; $TT - y = MNI - y \times 0.97 - 3.32$; $TT - z = MNI - y \times 0.05 + MNI - z \times 0.88 - 0.44$. Labels for brain activation foci were obtained in Talairach coordinates using the Talairach Daemon software, which provides accuracy similar to that of neuroanatomical experts.[26] The areas identified as labeled areas by this software were then confirmed by comparison with activation maps overlaid on MNI-normalized structural MRI images.

RESULTS

Clinical Background

As shown in Table 1, there were no statistically significant differences among the three groups in terms of gender, age, years of education, or score on Mini-Mental State Examination. There were no statistically significant differences between the two groups of depressive patients with respect to age at onset of first depressive episode, last episode-free period, score on Hamilton Rating Scale for Depression (HRSD), score on the Global Assessment of Functioning (GAF) scale, or dosage of antidepressants prescribed at the time of this study.

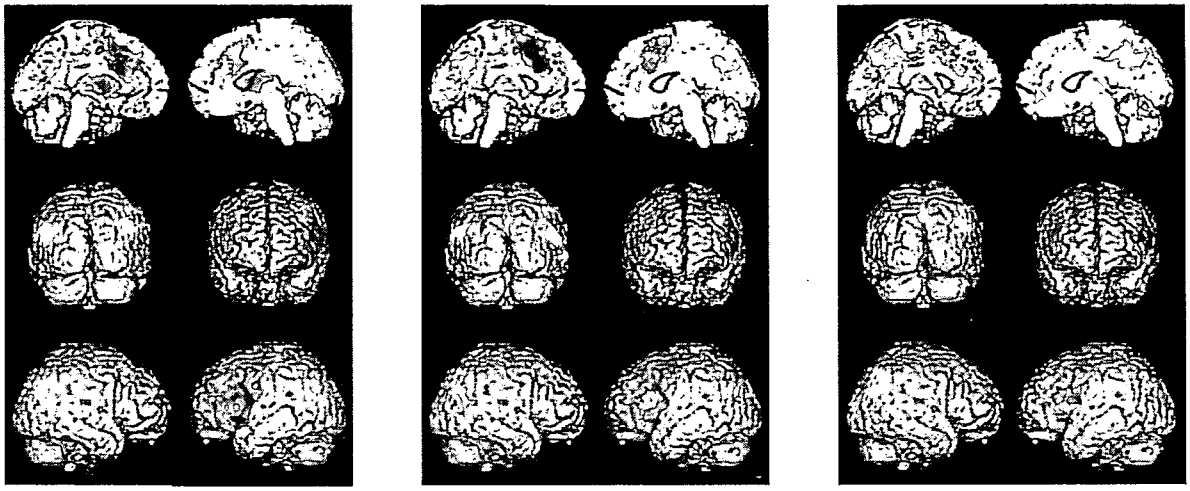
Behavioral Data

There was not a statistically significant between-group difference in the number of words generated in a verbal fluency task: single-episode group, 12.2 ± 2.5 words; multiple-episode group, 12.1 ± 3.2; comparison group, 11.9 ± 2.0 (ANOVA, $F=0.034$, $df=2,27$, $p=0.966$).

Neuroimaging Data

A one-sample *t*-test for each group revealed significantly increased activation in several brain regions involving the prefrontal cortex and anterior cingulate cortex, and significantly decreased activation in different several brain regions involving the parietal and temporal cortex (Figure 1, Table 2).

FIGURE 1. Statistical Parametric Maps of Brain Regions with Significant Activation and Deactivation During a Verbal Fluency Task in Each Three Groups



Comparison group | Single-episode group | Multiple-episode group

All activations were significant at a statistical threshold of p <0.001 (uncorrected) on the single-voxel level and p <0.05 (corrected) on the cluster level. Red scale represents increases and green scale decreases in activation. For exact coordinates, see Table 2. The images are projections of the significant points onto a normalized brain template.

Direct comparison by a two-sample *t*-test at each voxel of the brain activation among the three groups, showed that the comparison group had significantly greater activation than the multiple-episode group in the putamen, the left lateral globus pallidus, the anterior cingulate cortex and the right medial frontal cortex, and that the single-episode group had significantly greater activation than the multiple-episode group in the anterior cingulate cortex (Figure 2, Table 3). There were no significant differences in activation levels between the single-episode group and the comparison group at any brain region.

CONCLUSIONS

This is the first functional neuroimaging study undertaken to examine the relationship between number of previous depressive episodes in recovered depressed elderly and cerebral brain function. The major finding in this study was attenuated anterior cingulate activation in recovered depressed elderly patients with a previous history of multiple depressive episodes, compared with both those with a single episode and healthy volunteers. In contrast, there were no significant differences between patients with a single depressive episode and healthy volunteers.

To our knowledge, no study has reported a direct association between the number of prior depressive episodes and anterior cingulate function. However, two previous studies reported on the association between the number of depressive episodes and cognitive function. Driscoll et al.[8] reported that patients with late-onset, recurrent depression demonstrated more cognitive impairment on the Mattis Dementia Rating Scale compared to the late-onset, single-episode group. Also, Kessing[9] reported that the number of depressive episodes was correlated with cognitive decline on the Cambridge Cognitive Examination, the Global Deterioration Scale, and the like. Our major finding was attenuation of anterior cingulate cortex activation in depressed elderly with a history of multiple episodes of depression. Anterior cingulate cortex activation is related to the regulation of emotion and cognition,[27] and to the attentional demands of cognitive tasks such as the verbal fluency

TABLE 2. Areas With Significant Activation During a Verbal Fluency Task in Each Group

	Cluster Level		Voxel Level					
	Broadmann's Area	p Value	Number of Voxels in Cluster	p Value	T Score	X Coordinate	Y Coordinate	Z Coordinate
Comparison group[a]								
Left inferior frontal gyrus	47	0.000	20,051	0.009	20.21	−36	35	-4
Left middle frontal gyrus	46			0.009	20.06	−41	16	18
Left cingulate gyrus	24			0.012	19.05	−13	6	44
Right cerebellum		0.000	329	0.060	14.41	24	−63	−25
Right middle frontal gyrus	6	0.005	163	0.442	9.63	26	1	49
Right subgyral	6			0.989	6.39	19	1	53
Left fusiform gyrus	37	0.034	109	0.467	9.49	−41	−56	−10
Single-episode group[a]								
Left precuneus	7	0.002	246	0.034	15.15	−25	−63	33
Left superior frontal gyrus	6	0.000	8174	0.115	12.15	−6	10	51
Left medial frontal gyrus	32			0.126	11.95	−6	8	44
Left cingulate gyrus	24			0.154	11.49	−6	16	29
Right claustrum		0.000	732	0.416	9.27	33	8	−2
Right inferior frontal gyrus	47			0.528	8.69	31	22	−11
Multiple-episode group[a]								
Right claustrum		0.000	447	0.016	18.42	31	10	−3
Right inferior frontal gyrus	47			0.939	7.21	41	20	−6
Right insula	13			1.000	4.77	40	10	−5
Left claustrum		0.000	2,118	0.051	15.08	−31	10	0
Left insula	13			0.133	12.64	−40	3	19
Left precentral gyrus	6			0.169	12.06	−38	−3	28
Left superior frontal gyrus	6	0.000	1,227	0.097	13.40	−10	12	48
Left medial frontal gyrus	6			0.107	13.16	−1	−1	61
Left parahippocampal gyrus	36	0.035	103	0.412	9.95	−34	−29	−14
Left subgyral	20			0.990	6.48	−36	−13	−20
Right cingulate gyrus	24	0.001	197	0.634	8.82	19	−5	49
Right precentral gyrus	6			1.000	4.33	26	−15	48
Left middle occipital gyrus	19	0.001	205	0.657	8.72	−25	−81	15
Left lingual gyrus	17			0.966	6.92	−20	−83	3
Right cerebellum		0.000	470	0.748	8.30	27	−69	−28
Left fusiform gyrus	37	0.000	265	0.776	8.17	−36	−63	−7
Left cerebellum		0.002	186	0.791	8.09	−34	−60	−32

Notes: p values are corrected for spatial extent (cluster level p value) and peak height (voxel level p value) of the activation. All areas exceeding the corrected cluster level threshold of 0.05 are displayed. x, y, z coordinates: localization according to the standard Talairach coordinates (in mm).

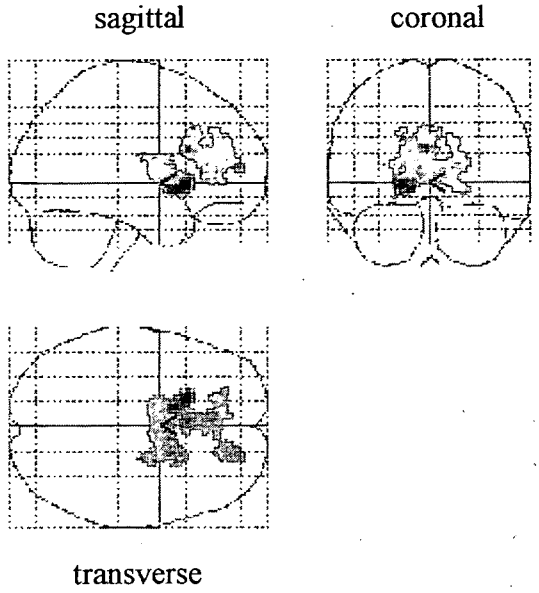
[a]One-sample *t*-test (df=9).

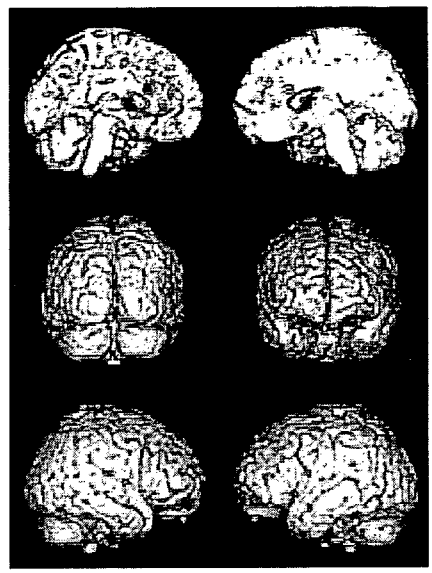
task used in this study.[28] Furthermore, this region is thought to play a central role in normalization of the cerebral dysfunction that accompanies recovery from depression.[29] Our results suggest that there might be an important association between attenuated anterior cingulate activation and the number of depressive episode in depressed elderly.

Most functional neuroimaging studies[10–17] in elderly patients with depression, as well as in depression generally,[30–32] have consistently reported global and regional decreases in cerebral perfusion and glucose metabolism, particularly in the prefrontal and anterior cingulate cortex, while some studies have showed different results of increases in the left lateral prefrontal cortex, the bilateral orbital frontal cortex, etc.[33–35] However, previous studies on recovered depressed elderly have provided inconsistent results. Reduced regional cerebral blood flow has been demonstrated to be remaining[11–12] or diminished[17] after clinical recovery. Matsuo et al.[15] reported that, in recovered depressed elderly, activation in the prefrontal cortex during a verbal fluency task was significantly less than in controls. This inconsistency may be due to study designs limitations, where there were no references to the number of prior depressive episode or where studies included patients with varying numbers of depressive episodes. The results of our study showed that in recovery the dysfunction

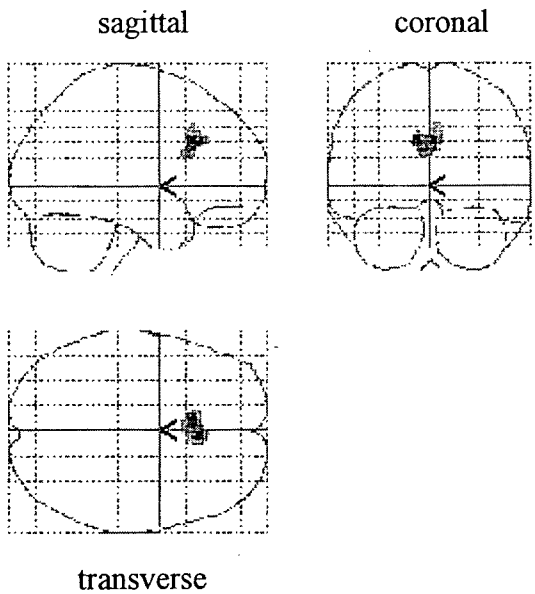
FIGURE 2. Statistical Parametric Maps of Brain Regions with Significant Activation Differences During a Verbal Fluency Task Among the Three Groups

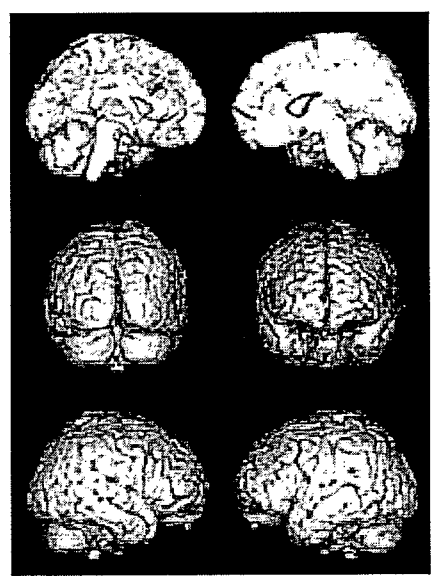
A. Comparison group > Multiple-episode group





B. Single-episode group > Multiple-episode group





[A] The comparison group had greater activation than the multiple-episode group. [B] The single-episode group had greater activation than the multiple-episode group. All activations were significant at a statistical threshold of $p < 0.001$ (uncorrected) on the single-voxel level and $p < 0.05$ (corrected) on the cluster level. For exact coordinates, see Table 3. The images on the left of each figure are through-projections onto representations of standard stereotactic space (sagittal, side view; coronal, view from back; transverse, view from above). The images on the right are projections of the significant points onto a normalized brain template.

TABLE 3. Areas With Significant Activation Differences Among the Three Groups During a Verbal Fluency Task

	Cluster Level		Voxel Level					
	Broadmann's Area	p Value	Number of Voxels in Cluster	p Value	T Score	X Coordinate	Y Coordinate	Z Coordinate
Increased more in comparison group than in multiple-episode group [a]								
Left putamen		0.000	1,229	0.006	8.71	−18	16	1
Left lateral globus pallidus				0.016	7.96	−11	6	−3
Right putamen				0.446	5.59	17	10	−3
Left anterior cingulate gyrus	33	0.000	793	0.091	6.77	−3	14	20
Left anterior cingulate gyrus	24			0.382	5.73	−6	30	7
Right medial frontal gyrus	10	0.016	202	0.238	6.09	15	47	13
Right anterior cingulate gyrus	32			0.968	4.48	15	35	12
Increased more in single-episode group than in multiple-episode group[a]								
Left anterior cingulate gyrus	32	0.034	195	0.555	5.28	−10	18	29
Right anterior cingulate gyrus	32			0.741	4.96	1	22	27
Left anterior cingulate gyrus	24			0.971	4.35	−4	16	22

Notes: No areas were indicated for increased more in multiple-episode group than in single-episode group, increased more in single-episode group than in comparison group, increased more in comparison group than in single-episode group, increased more in multiple-episode group than in comparison group. p values are corrected for spatial extent (cluster level p value) and peak height (voxel level p value) of the activation. All areas exceeding the corrected cluster level threshold of 0.05 are displayed. x, y, z coordinates: localization according to the standard Talairach coordinates (in mm).

[a]Two-sample *t*-test (df=18).

in anterior cingulate cortex was revealed not in patients with a single depressive episode but in patients with multiple episodes. Therefore, our results provide new evidence for an association between cerebral brain function and the cycling of depressive episodes in recovered depressed elderly. Another possible explanation of these findings may be the vulnerability to recurrence. However, from the present study, we cannot conclude both possibilities.

In addition to the anterior cingulate cortex, attenuated activation was found in the basal ganglia and the medial frontal cortex in patients with multiple depressive episodes compared with healthy volunteers, although there was no attenuation in activation of these areas compared with single-episode depression. These areas are included in the front subcortical pathways which appear to play an important role in depression.[30,36–38] The attenuated activation in the anterior cingulate cortex, basal ganglia and the medial frontal cortex might be jointly associated with the dysfunction of these front subcortical pathways and with a risk of recurrence of depression in the elderly.

There was one contradictory finding in our study, in that we failed to find a difference in the number of words generated on the verbal fluency task between the three groups, even though there were significant differences in regional brain function. However, this may be explained by the higher sensitivity of functional neuroimaging over behavioral measures for the measurement of cognitive processing.[39] Another explanation of this finding may be a possibility that the patients with multiple depressive episodes were able to produce comparable numbers of words by activating more brain regions other than anterior cingulate cortex compared to two other groups (Table 2).

Our study has some limitations. First, we did not perform a structured interview (such as Structured Clinical Interview for *DSM–IV*) during the selection of subjects for participation in the study. Nevertheless, their diagnoses were made cautiously by means of semistructured clinical interview according to *DSM–IV* criteria with two senior psychiatrists, review of the patients' hospital records, and discussions with their clinical psychiatrist. Second, our findings may be limited by potential medication effects. However, there was no significant difference between the two groups of patients with respect to the dosage of antidepressants prescribed at the time of this study. And no established study has reported a negative influence of antidepressant medications on brain activity in a verbal fluency task, and numerous studies have demonstrated

that administration of antidepressants is not associated with a decrease in cerebral blood flow.[40,41] Third, the multiple-episode group was 5.8 years younger at their first episode than the single-episode group without statistical significance. Thus any differences between the two groups might be due to some function of the age of first episode or its duration, for example.

In conclusion, our results show that the attenuated anterior cingulate cortex activation may be associated with multiple depressive episodes in recovered depressed elderly, and with the vulnerability to cycling or recurrence. However, whether that attenuated activation results in or from the multiple episodes remains unclear. To answer this question, further longitudinal studies using the same subjects with elderly depression would be required.

This work was supported by Grant-in-Aid for Research on Psychiatric and Neurological Diseases and Mental Health from Ministry of Health, Labor and Welfare; and Grant-in-Aid for Scientific Research from Ministry of Education, Culture, Sports, Science and Technology of Japan.

References

1. Reynolds CF, III, Frank E, Kupfer DJ, et al: Treatment outcomes in recurrent major depression: a post hoc comparison of elderly ("young old") and midlife patients. Am J Psychiatry 1996; 153: 1288-1292
2. Verhey FRJ, Honig A: Depression in the Elderly, in Depression: Neurobiological, Psychopathological and Therapeutic Advances. Edited by Honig A, van Praag HM. Chichester, John Wiley & Sons, 1997, pp 59-81
3. NIH Consensus Statement Update: Diagnosis and treatment of depression in late life. JAMA 1997; 278:1186-1190
4. Mitchell AJ, Subramaniam H: Prognosis of depression in old age compared to middle age: a systematic review of comparative studies. Am J Psychiatry 2005; 162:1588-1601
5. Greden JF: Antidepressant maintenance medications: when to discontinue and how to stop. J Clin Psychiatry 1993; 54:39-45
6. Baldwin RC, Simpson S: Prognosis and outcome studies in late-life depression. Clin Neurosci 1997; 4:16-22
7. Cole MG, Dendukuri N: Risk factors for depression among elderly community subjects: a systematic review and meta-analysis. Am J Psychiatry 2003; 160:1147-1156
8. Driscoll HC, Basinski J, Mulsant BH, et al: Late-onset major depression: clinical and treatment-response variability. Int J Geriatr Psychiatry 2005; 20:661-667
9. Kessing LV: Cognitive impairment in the euthymic phase of affective disorder. Psychol Med 1998; 28:1027-1038
10. Kumar A, Miller D: Neuroimaging in late-life mood disorders. Clin Neurosci 1997; 4:8-15
11. Smith GS, Reynolds CF, III, Pollock B, et al: Cerebral glucose metabolic response to combined total sleep deprivation and antidepressant treatment in geriatric depression. Am J Psychiatry 1999; 156:683-689
12. Nobler MS, Roose SP, Prohovnik I, et al: Regional cerebral blood flow in mood disorders, V.: effects of antidepressant medication in late-life depression. Am J Geriatr Psychiatry 2000; 8:289-296
13. de Asis JM, Stern E, Alexopoulos GS, et al: Hippocampal and anterior cingulate activation deficits in patients with geriatric depression. Am J Psychiatry 2001; 158:1321-1323
14. Matsuo K, Kato T, Fukuda M, et al: Alteration of hemoglobin oxygenation in the frontal region in elderly depressed patients as measured by near-infrared spectroscopy. J Neuropsychiatry Clin Neurosci 2000; 12:465-471
15. Matsuo K, Onodera Y, Hamamoto T, et al: Hypofrontality and microvascular dysregulation in remitted late-onset depression assessed by functional near-infrared spectroscopy. NeuroImage 2005; 26:234-242
16. Navarro V, Gasto C, Lomena F, et al: Frontal cerebral perfusion dysfunction in elderly late-onset major depression assessed by ^{99M}TC-HMPAO SPECT. NeuroImage 2001; 14:202-205
17. Navarro V, Gasto C, Lomena F, et al: Normalization of frontal cerebral perfusion in remitted elderly major depression: a 12-month follow-up SPECT study. NeuroImage 2002; 16:781-787
18. Warburton E, Wise RJ, Price CJ, et al: Noun and verb retrieval by normal subjects. Studies with PET. Brain 1996; 119:159-179
19. Oldfield RC: The assessment and analysis of handedness: the Edinburgh Inventory. Neuropsychologia 1971; 9:97-113
20. American Psychiatric Association: Diagnostic and Statistical Manual of Mental Disorders, 4th Ed. Washington DC, American Psychiatric Press, 1994
21. Inagaki A, Inada T: Dose equivalence of psychotropic drugs. Dose equivalence of novel antidepressants I. Jpn J Clin Psychopharmacol 2006; 9:1859-1864
22. Hamilton M: A rating scale for depression. J Neurol Neurosurg Psychiatry 1960; 23:56-62
23. Folstein MF, Folstein SE, McHugh PR: "Mini-mental state". a practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 1975; 12:189-198
24. Friston KJ, Holmes AP, Worsley KJ: How many subjects constitute a study? NeuroImage 1999; 10:1-5
25. Talairach P, Tournoux J: Co-planar stereotaxic atlas of the human brain. Germany, Thieme, 1998.
26. Lancaster JL, Woldorff MG, Parsons LM, et al: Automated Talairach atlas labels for functional brain mapping. Hum Brain Mapp 2000; 10:120-131
27. Bush G, Luu P, Posner MI: Cognitive and emotional influences in anterior cingulate cortex. Trends Cogn Sci 2000; 4:215-222
28. Phelps EA, Hyder F, Blamire AM, et al: fMRI of the prefrontal cortex during overt verbal fluency. Neuroreport 1997; 8:561-565
29. Mayberg HS, Brannan SK, Mahurin RK, et al: Cingulate function in depression: a potential predictor of treatment response. Neuroreport 1997; 8:1057-1061
30. Soares JC, Mann JJ: The functional neuroanatomy of mood disorders. J Psychiatr Res 1997; 31:393-432
31. Videbech P: PET measurements of brain glucose metabolism and

blood flow in major depressive disorder: a critical review. Acta Psychiatr Scand 2000; 101:11-20
32. Okada G, Okamoto Y, Morinobu S, et al: Attenuated left prefrontal activation during a verbal fluency task in patients with depression. Neuropsychobiology 2003; 47:21-26
33. Drevetz WC, Videen TO, Price JL, et al: A functional anatomical study of unipolar depression. J Neurosci 1992; 12:3628-3641
34. Biver F, Goldman S, Delvenne V, et al: Frontal and parietal metabolic disturbances in unipolar depression. Biol Psychiatry 1994; 36:381-388
35. Rosenberg R, Vorstrup S, Anderson A, et al: Effect of ECT on cerebral blood flow in melancholia assessed with SPECT. Convulsive Ther 1988; 4:62-73
36. Drevets WC: Neuroimaging studies of mood disorders. Biol Psychiatry 2000; 48:813-829
37. Sheline YI: Neuroimaging studies of mood disorder effects on the brain. Biol Psychiatry 2003; 54:338-352
38. Alexopoulos GS, Schultz SK, Lebowitz BD: Late-life depression: a model for medical classification. Biol Psychiatry 2005; 58:283-289
39. Goldberg TE, Weinberger DR: Genes and the parsing of cognitive processes. Trends Cogn Sci 2004; 8:325-335
40. Passero S, Nardini M, Battistini N: Regional cerebral blood flow changes following chronic administration of antidepressant drugs. Prog Neuropsychopharmacol Biol Psychiatry 1995; 19: 627-636
41. Ogura A, Morinobu S, Kawakatsu S, et al: Changes in regional brain activity in major depression after successful treatment with antidepressant drugs. Acta Psychiatr Scand 1998; 98: 54-59

Available online at www.sciencedirect.com
ScienceDirect
Behavioural Brain Research 186 (2008) 91–97
BEHAVIOURAL BRAIN RESEARCH
www.elsevier.com/locate/bbr

Research report

Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague–Dawley rats

A. Imanaka, S. Morinobu*, S. Toki, S. Yamamoto, A. Matsuki, T. Kozuru, S. Yamawaki

Department of Psychiatry and Neurosciences, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, 1-2-3 Kasumi, Minami-ku, 734-8551 Hiroshima, Japan

Received 19 March 2007; received in revised form 23 July 2007; accepted 28 July 2007
Available online 6 August 2007

Abstract

It is well known that early life events induce long-lasting psychophysiological and psychobiological influences in later life. In rodent studies, environmental enrichment after weaning prevents the adulthood behavioral and emotional disturbances in response to early adversities. We compared the behavioral effect of neonatal isolation (NI) with the effect of NI accompanied by tactile stimulation (NTS) to determine whether NTS could reverse or prevent the effects of NI on the adulthood behavioral and emotional responses to environmental stimuli. In addition, we also examined the sex difference of the NTS effect. Measurements of body weights, an open-field locomotor test, an elevated plus maze test, a hot-plate test, and a contextual fear-conditioning test were performed on postnatal day 60. As compared with rats subjected to NI, rats subjected to NTS showed significantly higher activity and exploration in the open-field locomotor test, lower anxiety-like behavior in the elevated plus maze test, and significantly prolonged latencies in the hot-plate test, and this effect was equal among males and females. In the contextual fear-conditioning test, whereas NTS significantly reduced the enhanced freezing time due to NI in females, no significant difference in the freezing time between NI and NTS was found in males. These findings indicate that adequate tactile stimulation in early life plays an important role in the prevention of disturbances in the behavioral and emotional responses to environmental stimuli in adulthood induced by early adverse experiences.

Keywords: Neonatal isolation (NI); Neonatal tactile stimulation (NTS); Rat; Open-field locomotor test; Elevated plus maze test; Hot-plate test; Contextual fear-conditioning test

1. Introduction

It is well known that early experiences induce long-lasting psychophysiological and psychobiological changes in later life. Numerous studies have demonstrated that early adverse experiences such as maternal separation (MS) or neonatal isolation (NI), which interrupt dam–pup interactions, affect the development of the central nervous system and subsequently lead to enhanced susceptibility to stress in adulthood, both behaviorally and endocrinologically [8,10,12,14,17,37]. NI and MS differ with respect to isolation of individual offspring. With most MS procedures, only the dams are removed to separate cages, while the pups remain in their home cage. With NI procedures, pups are isolated and placed individually into containers separately from their dams and littermates. In contrast, the protective or therapeutic effects of early intervention on the development of stress vulnerability during the interruption of the dam–pup relationship has not been as thoroughly examined. For example, brief handling of neonatal rats during maternal separation was reported to induce resistance of the hypothalamo-pituitary-adrenal (HPA) axis to stress in adult rats [17]. Several studies have demonstrated that adult rats subjected to neonatal handling exhibited less anxiety-like behaviors in the elevated plus maze as compared with nonhandled rats [19,27].

Another type of neonatal handling, neonatal tactile stimulation (NTS), also has a distinct effect on the development of stress reactivity. Rats subjected to NTS show increased curiosity and problem-solving ability, and exhibit less emotionality in stressful situations [16]. NTS prevents the rise of serum corticosterone

Abbreviations: NI, neonatal isolation; NTS, neonatal tactile stimulation

* Corresponding author. Tel.: +81 82 257 5205; fax: +81 82 257 5209.
E-mail address: smoriob@hiroshima-u.ac.jp (S. Morinobu).

0166-4328/$ – see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbr.2007.07.039

levels typically associated with maternal deprivation stress and causes elevated corticosterone levels to return to normal [25]. NTS also protects against maternal deprivation-induced shortening of hot-plate latencies [35]. Furthermore, it has been reported that NTS alleviates the reduction of hippocampal volume in rats subjected to neonatal hypoxia–ischemia [29]. These findings indicate that subjecting animals to NTS can facilitate their ability to cope with stressful environmental conditions, decrease the enhanced HPA axis in response to stress in adulthood, and protect against brain damage induced by neonatal manipulation.

Postnatal handling, which involves only a brief period (15 min) of mother–pup separation, dampens HPA responses to stress [15,20,21]. In contrast, postnatal MS (3 h/day; PN days 2–14) or (6 h/day; PN days 2–10) enhances HPA responses to stressors [18,28]. In addition, 1 h-neonatal isolation on postnatal days 2–9 also enhances HPA responses to stressors [3]. It has also been shown that early adverse experiences have sex-specific effects on the development of HPA-axis reactivity [4,36]. Similarly, gender differences exist with respect to the effects of neonatal isolation and neonatal handling on the development of anxiety-like behavior in the elevated plus maze [8,19] and the conditioned fear test [1,11]. Although there was no significant sex differences in anxiety-like behavior between rats subjected to neonatal handling and neonatal handling with tactile stimulation [32], it is unclear whether sex differences exist with respect to the ability of NTS to prevent or reverse the enhancement of susceptibility to environmental stimuli in response to early adversities.

In this context, the present study was undertaken to assess whether NTS can prevent or reverse the enhanced susceptibility to environmental stimuli due to NI in adult rats. We compared the effect of NI with the effect of NTS during NI on body weights, locomotor activities in the open-field test, anxiety-like behavior in the elevated plus maze test, pain sensitivity in the hot-plate test, and the fear responses in a contextual fear test on postnatal day 60. We also examined sex-specific effects of NI and NTS on these behavioral tests.

2. Materials and methods

2.1. Animals

Pregnant female Sprague–Dawley rats were purchased from Charles River (Yokohama, Japan). The rats were housed individually in the breeding colony at constant room temperature (23 ± 2 °C) and humidity (60%) with a 12/12 h light–dark cycle (lights on at 08:00). Food (Rodent Lab Diet EQ 5L37, Japan SLC Inc.) and water, conforming to the Water Quality Standard required by the Japanese Waterworks Law, were provided ad libitum. Male (n = 153) and female (n = 151) SD rats were used, and no more than two pups from the same dam were used in behavioral experiments. The experimental animals were divided into the following groups: (1) sham-treatment, (2) NI, and (3) NTS. Prior to birth, litters from each dam were randomly assigned to the sham, NI, and NTS groups. Litters were weaned on postnatal (PN) day 22. After weaning, male and female rats were housed in same-sex, same-treatment groups of three per cage (38 cm × 23 cm × 20 cm stainless steel cage) and maintained under normal conditions until the behavioral experiments; these included the open-field locomotor test (males; sham: n = 10, NI: n = 10, NTS: n = 10, females; sham: n = 10, NI: n = 10, NTS: n = 10), elevated plus maze test (males; sham: n = 12, NI: n = 16, NTS: n = 12, females; sham: n = 12, NI: n = 13, NTS: n = 15), hot-plate test (males; sham: n = 12, NI: n = 12, NTS: n = 15, females; sham: n = 12, NI: n = 11, NTS: n = 12), and contextual fear-conditioning test (males; sham: n = 14, NI: n = 14, NTS: n = 16, females; sham: n = 15, NI: n = 16, NTS: n = 15), which were undertaken on PN day 60 (Fig. 1). A different set of rats was used for each of these experiments. All animal procedures were approved by the Hiroshima University Medical Science Animal Care Committee.

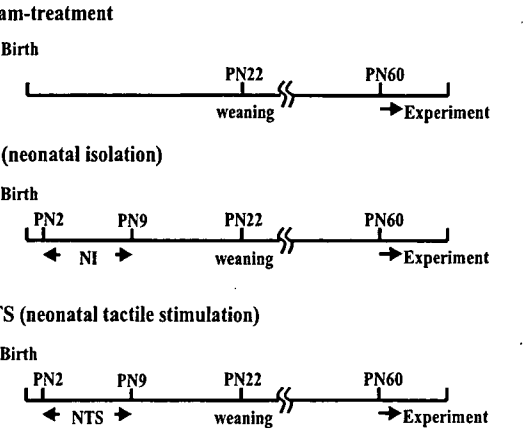


Fig. 1. Animal treatment paradigms. Prior to birth, litters from each dam were randomly assigned to (A) sham, (B) NI, and (C) NTS groups depending on neonatal treatments. All litters were weaned on postnatal (PN) day 22. After weaning, male and female rats in all groups were housed in same-sex, same-treatment groups of three per cage. Behavioral experiments were performed on PN day 60.

2.2. Neonatal isolation (NI)

After birth, the pups and mothers were housed together in their home cages (38 × 23 × 20 cm clear plastic cages) until weaning. Kehoe and Bronzino's method [9] was used for NI treatment. The first 24-h period after birth was designated PN day 1. Only litters with 11–14 pups were used in this study, and there were no differences in mean litter size among the three groups (NI, NTS, sham-treatment). The number of male and female pups was equal or almost equal in each litter (e.g., five males, six females). In the NI group, pups were isolated from the dam, nest, and siblings, and placed in individual opaque round containers (7 cm diameter and 8 cm deep) without bedding in a temperature- and humidity-controlled chamber, for 1 h per day on PN days 2–9. This microenvironment temperature was 30 ± 2 °C, similar to nest temperature, and humidity was 60%. Containers were placed 20 cm apart. Isolation was carried out between 09:00 and 12:00 each day. The rats in the sham group was housed under normal conditions and left undisturbed, except for weekly cage cleaning, until weaning. The rats in the sham group were similar to what are usually designated as animal facility-reared (AFR) animals.

2.3. Neonatal tactile stimulation (NTS)

Pups were isolated from the dam, nest, and siblings, and placed in individual round containers, as described above for NI. All pups were then gently handled dorsally from head to tail for 1 h per day by an investigator whose hands were covered with fine latex gloves. After handling, all pups were returned to the home cage at the same time. The duration of each handling session was approximately 30 s per pup and each pup was handled for a total of 5 min. This procedure was conducted on PN days 2–9.

2.4. Body weight

Body weight (g) was measured on the day of weaning (PN day 22), PN day 40, and on PN day 60.

2.5. Behavioral studies

All behavioral experiments were undertaken on PN day 60, and the animals were left without handling in the home cage (38 cm × 23 cm × 20 cm stainless steel cage), except for the measurement of body weight on PN day 40 until testing started. Rats were tested between 08:00 and 12:00. All behavioral data were collected by blind observers who were seated inside the testing room.

2.5.1. Open-field locomotor test

In the open-field locomotor test, rats were placed at the centre of a cubic chamber (48 cm × 48 cm × 48 cm). The animal's horizontal movements, measured by automatic actography (SCANET MV-10; Melquest, Toyama, Japan), were estimated as the number of interruptions of the near infrared rays. The infrared sensors were set at a height of 10 cm from the floor, and the distance between the photo beams was 6 mm. Additionally, the number of rearings (standing upright on the hind legs) was also measured. All animals were habituated to the testing room for 20 min before the start of each session and the testing sessions lasted for 5 min. The open-field area was cleaned with 10% ethanol and then with water between each experiment. The test room was dimly illuminated with indirect white lighting.

2.5.2. Elevated plus maze test

The plus maze consisted of two open (50 cm × 10 cm) and two closed (50 cm × 10 cm × 38 cm) arms, arranged perpendicularly, and was elevated 73 cm above the floor. Each rat was placed in the center of the apparatus and the number of entries and time spent per open and closed arms was recorded via a video camera mounted above the center of the apparatus. Each rat was habituated to the testing room for at least 20 min before being placed in the center of the maze. The apparatus was cleaned with alcohol after each rat was tested. The trial lasted for 5 min, after which the rat was removed from the maze and returned to its home cage. The test room was dimly illuminated with indirect white lighting. The duration of time spent in arms, the number of visits to arms, the percentage of time in the open arms (time in the open arms divided by the time in both arms), the percentage of open arm entries (number of entries into the open arm divided by total number of entries in both arms), total entries (the number of open plus closed arm entries), and head dippings from the open arm (protrusion of the head over the edge of an open arm) were evaluated.

2.5.3. Hot-plate test

The hot-plate test is one of the most commonly used methods for determining analgesic efficacy in rodents. Each rat was placed in a glass beaker on a hot-plate (HPT-1; Melquest, Toyama, Japan). A hot-plate analgesia meter, maintained at 52.5 °C, was used for this experiment. Latency to flinch or raise hind paws was recorded. To prevent tissue damage, the rat was removed from the hot plate if it did not respond within 30 s [34].

2.5.4. Contextual fear-conditioning test

Fear-conditioning tests were performed as follows: The conditioning chamber was located in a windowless room and housed in a soundproof box (70 cm × 60 cm × 60 cm). The conditioning chamber (50 cm × 28 cm × 32.5 cm) was made of transparent acrylic resin on three sides and aluminum on the other two. One of the metal sides had a speaker and three 24 V lights. A clear plexiglass window allowed the rat to be continually observed. The chamber was equipped with an 18-bar insulated shock grid floor. The floor was removable, and between tests the floor and interior of the chamber were cleaned with 70% ethanol and then with water. Each bar (5 mm in diameter) was connected through a harness to a shock generator-scrambler (Model SGS003; Muromachi, Tokyo, Japan), a device that delivers scrambled shock. Only one rat at a time was present in the experimental room. The other rats remained in their home cages. Each rat was carried to the behavioral room in a fresh cage that was identical to the home cage.

For the contextual conditioning experiments, rats were placed in the conditioning chamber 180 s before the onset of the unconditioned stimuli (US) (continuous foot shock at 0.8 mA for 4 s). After the test, rats were placed back in their home cages. Twenty-four hours later, rats were placed again in the same conditioning chamber and contextual freezing was assessed. Conditioning was assessed based on measurements of freezing, defined as the total absence of body and head movement, except for that associated with breathing. Freezing behavior of the rat was recorded using a video recorder for 180 s after the onset of the conditioned stimuli, and later scored blindly by the experimenter. Fear was quantified as the amount of time (in s) spent freezing.

2.6. Statistical analysis

Behavioral parameters were expressed as the mean (±S.E.M.). Statistical analysis was performed by two-way analysis of variance (ANOVA) to compare the means in different groups of each gender. One factor was neonatal treatment (sham-treatment or NI, or NTS), and another factor was gender (male or female). In case there were no gender differences, we analyzed treatment effects with pairing of gender on littermates. Tukey's test was used for multiple comparisons. Statistical significance between groups was set at $p < 0.05$.

3. Results

3.1. Body weight

Mean body weights of the groups on PN day 22 (weaning day), PN day 40, and PN day 60 are presented in Table 1. While two-way ANOVA revealed a significant effect of gender [$F(1, 144) = 8.799, p < 0.05$], [$F(1, 144) = 1300.440, p < 0.0001$], [$F(1, 144) = 2150.624, p < 0.001$], on PN days 22, 40, and 60, respectively, there was no significant effect of neonatal treatment nor an interaction between neonatal treatment and gender. On weaning day, the mean body weight of females was significantly greater than that of males. In contrast, the mean body weight of males was significantly greater than that of females in adulthood.

3.2. Open-field locomotor test

In the open-field locomotor test, two-way ANOVA revealed significant effects of neonatal treatment [$F(2, 54) = 16.353$, $p < 0.0001$], and gender [$F(1, 54) = 6.911, p < 0.05$] on horizontal movements. No significant interaction between neonatal treatment and gender [$F(2, 54) = 2.570$, $p = 0.186$] was found. Post hoc comparison revealed that the mean number of horizontal movements of rats in the NTS group was significantly higher

Table 1
Body weights (g) on the day of weaning (PN day 22), 40, and 60 of Sprague–Dawley (SD) male and female rats (sham, NI, NTS)

Group	Weaning (PN22)	PN40	PN60
Male			
Sham	51.4 ± 1.8	242.8 ± 1.9*	341.6 ± 3.7
NI	48.8 ± 2.3	242.1 ± 3.9*	337.8 ± 3.0$^{\$}$
NTS	52.8 ± 1.1	245.1 ± 2.4*	339.8 ± 3.8$^{\$}$
Female			
Sham	54.5 ± 1.6$^{\#}$	165.2 ± 2.8	205.1 ± 4.4
NI	53.7 ± 0.9$^{\#}$	164.4 ± 2.9	208.3 ± 2.6
NTS	55.8 ± 1.0$^{\#}$	166.4 ± 1.5	212.4 ± 2.9

Each group consisted of male ($n = 25$/group) and female ($n = 25$/group) rats. Values were expressed as the means ± S.E.M. On weaning day (PN 22), females exhibited significant greater body weight than males. Males demonstrated significant greater body weight than females on PN 40 and 60, $^{\#}p < 0.05$ compared with males (PN 22). $^{*}p < 0.0001$ compared with females (PN 40). $^{\$}p < 0.001$ compared with females (PN 60).

Table 2
Effects of two different neonatal treatments on locomotor activity in adult rats

	Sham	NI	NTS
(a) Horizontal movements (counts/5 min)			
Male	2139.7 ± 106.7	2098.4 ± 103.2	2549.2 ± 150.2*
Female	2054.4 ± 94.8#	1602.3 ± 58.2#	2440.8 ± 134.4*,#
(b) Rearing			
Male	16.9 ± 2.1	12.0 ± 1.9$	27.8 ± 2.7$,&
Female	15.8 ± 0.8	10.2 ± 0.9$	24.6 ± 1.2$,&

The levels of horizontal locomotor activity (a), and rearing (b), among groups in the open-field locomotor test. Each group consisted of male ($n = 10$/group) and female ($n = 10$/group) rats. Data were expressed as the means ± S.E.M. *$p < 0.0001$ compared with the NI group. #$p < 0.05$ compared with males. $$p < 0.05$ compared with the sham group. &Indicates a significant difference ($p < 0.0001$) between the NI and the NTS groups.

than that of rats in the NI group. The mean number of horizontal movements of female rats was lower than that of male rats (Table 2a).

Two-way ANOVA revealed a significant effect of neonatal treatment on rearing [$F(2, 54) = 38.335$, $p < 0.0001$], but no significant of gender [$F(1, 54) = 2.655$, $p = 0.109$] and no significant interaction between neonatal treatment and gender [$F(2, 54) = 0.303$, $p = 0.740$]. Post hoc comparison showed that the mean rearing of the NTS group was significantly higher than that of the other two groups, and that was significantly lower in the NI group than in the sham group (Table 2b).

3.3. Elevated plus maze test

For the elevated plus maze test, two-way ANOVA revealed a significant effect of neonatal treatment [$F(2, 74) = 27.919$, $p < 0.0001$], but no significant effect of gender [$F(1, 74) = 1.604$, $p = 0.209$] and no significant interaction between neonatal treatment and gender [$F(2, 74) = 0.556$, $p = 0.576$] on the duration of time spent in open arms. Post hoc comparison showed that the duration of time spent in open arms in the NTS group was significantly longer than in the sham and NI groups and was significantly shorter in the NI group than in the sham group. Two-way ANOVA of data on the duration of time spent in closed arms revealed a significant effect of neonatal treatment [$F(2, 74) = 6.310$, $p < 0.001$] but no significant effect of gender [$F(1, 74) = 0.990$, $p = 0.323$] and no significant interaction between neonatal treatment and gender [$F(2, 74) = 0.453$, $p = 0.637$]. Post hoc comparison showed that the duration of time spent in closed arms in the NTS group was significantly shorter than in the NI group (Table 3).

Two-way ANOVA of data on the number of visits to open arms revealed significant effects of neonatal treatment [$F(2, 74) = 28.100$, $p < 0.0001$], and gender [$F(1, 74) = 10.250$, $p < 0.001$], but there was no significant interaction between these variables [$F(2, 74) = 0.665$, $p = 0.517$]. Post hoc comparison showed that the number of visits to open arms in the NTS group was significantly higher than in the sham and NI groups, and significantly lower in the NI group than in the sham group. The mean number of visits to open arms for female rats was lower than that for male rats. For the number of visits to closed arms, two-way ANOVA revealed a significant effect of gender [$F(1, 74) = 33.360$, $p < 0.0001$] but no significant of neonatal treatment [$F(2, 74) = 0.747$, $p = 0.477$] and no significant interaction between neonatal treatment and gender [$F(2, 74) = 3.728$, $p = 0.069$]. Post hoc comparison showed that the mean number of visits to closed arms for female rats was lower than that for male rats (Table 3).

On the percentage of time spent in the open arms (% open arm time), two-way ANOVA revealed a significant effect of neonatal treatment [$F(2, 74) = 24.550$, $p < 0.0001$] but no significant of gender [$F(1, 74) = 0.156$, $p = 0.694$] and no significant interaction between neonatal treatment and gender [$F(2, 74) = 0.589$, $p = 0.557$]. Post hoc comparison showed that the % open arm time in the NTS group was significantly higher than in the sham and NI groups. The % open arm time was significantly lower in the NI group than in the sham group. Similarly, two-way ANOVA revealed a significant effect of neonatal treatment [$F(2, 74) = 22.979$, $p < 0.0001$], no significant of gender [$F(1, 74) = 0.427$, $p = 0.515$] and no significant interaction between

Table 3
Effects of two different neonatal treatments on explorative and anxiety-like behavior in adult rats

	Male			Female		
	Sham	NI	NTS	Sham	NI	NTS
Duration of time spent in arms (s)						
Open arms	55.7 ± 4.1	48.4 ± 5.4*	102.4 ± 6.5*,#	77.4 ± 5.1	42.8 ± 4.4*	107.7 ± 8.4*,#
Closed arms	156.6 ± 6.1	169.1 ± 7.8	138.8 ± 9.6#	171.1 ± 8.8	186.7 ± 8.3	135.6 ± 9.4#
Number of visits spent in arms						
Open arms	8.9 ± 0.6	6.5 ± 0.5*	10.7 ± 1.0*,#	7.3 ± 0.8&	3.7 ± 0.5*,&	9.6 ± 0.6*,#,&
Closed arms	8.8 ± 0.5	8.3 ± 0.5	7.5 ± 0.4	5.7 ± 0.4&	5.1 ± 0.3&	6.7 ± 0.5&
%open arm time	26.2 ± 1.7	22.9 ± 2.8*	42.7 ± 4.6*,#	31.3 ± 3.2	18.1 ± 1.7*	43.9 ± 3.8*,#
%open arm entries	50.2 ± 3.3	42.9 ± 2.9*	57.8 ± 3.6*,#	54.8 ± 2.9	41.1 ± 3.7*	59.2 ± 2.8*,#
Total entries	17.7 ± 0.8$	14.8 ± 0.9	18.2 ± 1.4$	12.9 ± 1.2$,&	8.9 ± 1.1&	16.3 ± 0.9$,&
Head dippings	7.0 ± 0.6$	4.1 ± 0.4	10.5 ± 0.5$	6.8 ± 0.4$	4.8 ± 0.7	8.5 ± 0.4$

Comparison of the duration of time spent in arms, the number of visits to arms, the percentage of time in the open arms (%open arm time), the percentage of open arm entries (%open entries), total entries, head dippings from the open arm, among groups in the elevated plus maze test. Each group consisted of male (sham: $n = 12$, NI: $n = 16$, NTS: $n = 12$) and female (sham: $n = 12$, NI: $n = 13$, NTS: $n = 15$) rats. Data were expressed as the means ± S.E.M. *$p < 0.05$ compared with the sham group, #indicates a significant difference ($p < 0.0001$) between the NI and the NTS groups, $$p < 0.05$ compared with the NI groups, &$p < 0.05$ compared with males.

neonatal treatment and gender [$F(2, 74) = 0.824$, $p = 0.443$] on the percentage of entries in the open arms (% open arm entries) (Table 3).

Locomotion in the elevated plus maze was reflected by the total number of entries (total entries). While two-way ANOVA revealed significant effects of neonatal treatment [$F(2, 74) = 16.074$, $p < 0.0001$], and gender [$F(1, 74) = 27.119$, $p < 0.0001$], there was no significant interaction between these variables [$F(2, 74) = 2.309$, $p = 0.106$]. Post hoc comparison revealed that the mean number of total entries was significantly lower in the NI group than in the sham and NTS groups. The mean number of total entries for female rats was lower than that for male rats (Table 3).

Head dipping in the elevated plus maze test is an exploratory behavior. Two-way ANOVA revealed a significant effect of neonatal treatment [$F(2, 74) = 56.056$, $p < 0.0001$], no significant of gender [$F(1, 74) = 1.534$, $p = 0.219$], and a significant interaction between neonatal treatment and gender [$F(2, 74) = 3.849$, $p < 0.05$]. Post hoc comparison revealed that the mean number of head dippings was significantly lower in the NI group than in the sham and NTS groups (Table 3).

3.4. Hot-plate test

In the hot-plate test, two-way ANOVA revealed significant effects of neonatal treatment [$F(2, 68) = 44.326$, $p < 0.0001$], and gender [$F(1, 68) = 4.657$, $p < 0.05$] on latency to flinch or raise hind paws. No significant interaction between neonatal treatment and gender [$F(2, 68) = 1.270$, $p = 0.287$] was found. Post hoc analysis revealed that the latency was significantly longer in the NTS group than in the other two groups, and that the latency was significantly shorter in the NI group than in the sham group. The latency for female rats was shorter than that for male rats (Table 4).

3.5. Contextual fear-conditioning test

In the contextual fear-conditioning test, two-way ANOVA revealed significant effects of neonatal treatment [$F(2, 84) = 15.636$, $p < 0.0001$] and gender [$F(1, 84) = 9.934$, $p < 0.01$], and a significant interaction between neonatal treatment and gender [$F(2, 84) = 6.710$, $p < 0.01$] on contextual freezing time. Post hoc comparison revealed that the contextual freezing time was significantly lower in NTS males than in sham males. The contextual freezing time of NI males tended to be significantly lower than that of sham males ($p = 0.09$).

Table 4
Effects of two different neonatal treatments on pain sensitivity in adult rats

	Sham	NI	NTS
Male	12.5 ± 1.3	11.0 ± 0.7*	15.6 ± 2.6*,#
Female	10.8 ± 1.8$	9.5 ± 0.9*,$	15.7 ± 2.2*,#

Comparison of latency (s) to flinch or raise hind paws among groups in the hot-plate test. Each group consisted of male (sham: $n = 12$, NI: $n = 12$, NTS: $n = 15$) and female (sham: $n = 12$, NI: $n = 11$, NTS: $n = 12$) rats. Data were expressed as the means ± S.E.M. *$p < 0.05$ compared with the sham group, #$p < 0.05$ compared with the NI group, $$p < 0.05$ compared with males.

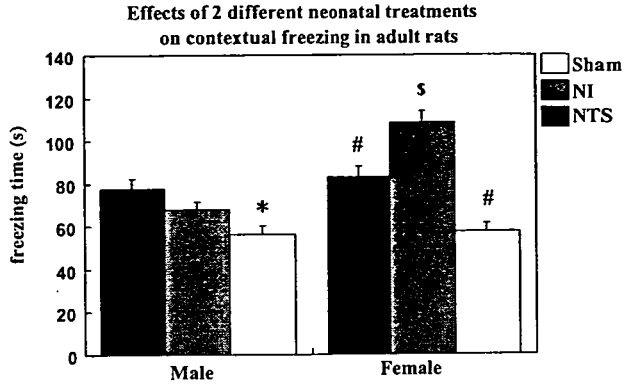


Fig. 2. Effects of two different neonatal treatments on contextual freezing time (s) in adult rats during a 180-s interval. Each group consisted of male (sham: $n = 14$, NI: $n = 14$, NTS: $n = 16$) and female (sham: $n = 15$, NI: $n = 16$, NTS: $n = 15$) rats. The results were expressed as the mean ± S.E.M. (*) indicates a significant difference ($p < 0.05$) between sham males and NTS males; (#) $p < 0.05$ compared with NI females; ($) indicates a significant difference ($p < 0.0001$) between NI males and NI females.

The enhancement of contextual freezing time was significantly greater in NI females than in sham or NTS females. NTS females showed significantly less contextual freezing than sham females. Surprisingly, contextual freezing time was enhanced more in NI females than in NI males (Fig. 2).

4. Discussion

In the present study, we examined how two different types of neonatal manipulations (NI as an adverse experience versus NTS as an intervention for adverse experience) in rats affect development, locomotor and exploratory behavior, anxiety-like behavior, pain sensitivity, and contextual fear in adulthood. Furthermore, we examined the sex-specific effect of neonatal manipulations on the susceptibility to environmental stimuli in adulthood. This study had three major findings: (1) Neither NI nor NTS affects gross physical development. (2) Among both sexes, NTS can reverse the decrease in rearing in the open-field locomotor test, the enhanced anxiety-like behavior in the elevated plus maze test, and the increased pain sensitivity in the hot-plate test of rats subjected to NI in adulthood. In most of these behavioral tests, NTS can promote behavioral and emotional responses compared to rats subjected to sham-treatment. The rats subjected to NTS showed increased rearing, decreased anxiety-like behavior, pain sensitivity, and contextual freezing to compare with those subjected to sham-treatment. (3) In the contextual fear-conditioning test in adulthood, there is a significant interaction between neonatal treatments and gender. NTS can reverse the enhanced fear induced by NI in female but not male rats. Overall, the results of the present study of NI indicated that this neonatal treatment reduced rearing and explorative behaviors, and enhanced anxiety-like behavior among both sexes. These findings are in agreement with a previous report demonstrating that maternally separated rats showed higher levels of anxiety and fear than sham-treated rats in adulthood, as measured by the open-field test and elevated plus maze test [8].