could be a less-invasive surgery with a short hospital stay that would allow patients with intractable Meniere's disease to return to work as soon as possible.

MATERIALS AND METHODS

Patients. The study included 50 patients, including cases from our previous preliminary report,[24] who were cared for at our hospitals (Osaka Rosai Hospital and Osaka University Hospital) between April 1, 1998, and March 31, 2002, had a definitive diagnosis of Meniere's disease according to the criteria presented by the Health Science Research Grant for Specific Disease of the Ministry for Health and Welfare, Japan,[25] and underwent EDSS. There were 8 bilateral cases and 42 unilateral cases; 17 unilateral cases had intact canals and 25 had deteriorated canals. All of these patients were considered to have intractable Meniere's disease after various medical management strategies had failed at least 6 months before operation. Before surgery, we obtained informed consent for EDSS. The patients averaged 45.0 ± 2.0 years of age. They had been under observation an average of 50.9 ± 6.3 months and had had vertigo an average of 2.09 ± 0.2 times per month for the past 6 months. The average hearing threshold was 57.2 ± 2.8 dB. In the 42 patients with unilateral Meniere's disease, the average score on the preoperative caloric test was 28.3% ± 4.1%.

Surgical Procedures. The technical details of EDSS were performed as described previously.[23] Briefly, we performed a simple mastoidectomy, clearly exposing the endolympatic sac in the area between the sigmoid sinus and the inferior margin of the posterior semicircular canal. The sac was opened with an L-shaped or backward L–shaped incision made along the posterior and distal margins of the lateral wall. The sac was then filled with a 20-mg mass of prednisolone. While the mass dissolved in the sac, we prepared a bundle of absorbable gelatin films (5 sheets) with fan-shaped and stick-shaped ends. These films were tied to each other with biochemical adhesive (human thrombin combined with human fibrinogen) at the stick-shaped end only. The fan-shaped end was then inserted into the sac, and small pieces of absorbable gelatin sponge soaked in a high concentration of dexamethasone sodium phosphate (32 mg/4 mL) were placed inside and outside the sac lumen expanded with the bundle. The sponges containing dexamethasone placed outside the sac were coated with the adhesive so that dexamethasone was slowly delivered into the sac over a long period of time by means of a natural sustained-release vehicle. The stick-shaped end of the gelatin film bundle extending out of the sac was fixed to the front edge of the mastoid cavity with the same adhesive, so that the incision into the sac was expanded for an adequate period of time after surgery. The mastoid cavity was filled with relatively large pieces of absorbable gelatin sponge dipped in antibiotic solution, and the wound was closed with skin sutures.

Patients were observed after the operation until the complete disappearance of subjective and objective symptoms. Patients were instructed to remain in bed until the day after the operation; they were then allowed to walk within the hospital. In addition, patients were prescribed only antibiotics and anti–gastric ulcer agents during a given period of time after the operation, and they were not given antidizziness or antianxiety agents.

Examinations. We asked patients about subjective vestibular symptoms (static and motion-evoked dizziness) every day or every other day after the operation and recorded their responses during the period of convalescence. Furthermore, we observed objective vestibular findings (spontaneous, positional, and positioning, ie, Dix-Hallpike, nystagmus) using ENG during the period of convalescence.

As possible factors influencing the status of dizziness during the convalescence period, we examined 1) the age at operation (average, 45.0 ± 2.0 years); 2) the affected duration before surgery (average, 50.9 ± 6.3 months); 3) the average number of definitive spells of vertigo per month during the 6 months before surgery (average, 2.09 ± 0.2); 4) the worst hearing level in 6 months before surgery (average, 57.2 ± 2.8 dB); and 5) the score of the preoperative caloric test in the 42 patients with unilateral disease (average, 28.3% ± 4.1%). Concerning the caloric test, 20 mL of 30°C (cold) water and 20 mL of 44°C (hot) water were injected by turns into the external auditory meatus for 10 seconds, and the induced nystagmus was recorded by ENG under dark, open-eyes conditions. The CP scores were based on the maximum slow phase eye velocity, and values of more than 25% were recognized as positive. (A positive score means the operated side is dominant.) The caloric test was conducted in all patients except for the 8 who had bilateral symptoms.

Statistical Analysis. The significance of the relationships between the background factors mentioned above and the durations of subjective and objective vestibular symptoms during convalescence after surgery were examined by the Pearson test. The significance of the differences of the durations of symptoms between bilaterally and unilaterally affected cases or between unilateral cases with intact and deteriorated canals was examined by the *t*-test. In all statistical analyses, a p level of less than .05 was considered to be significant.

DURATION (IN DAYS) OF VESTIBULAR SYMPTOMS AFTER SURGERY

	Subjective Sensations		*Objective Findings*		
	Static Dizziness	*Motion-Evoked Dizziness*	*Spontaneous Nystagmus*	*Positional Nystagmus*	*Positioning Nystagmus*
All cases (n = 50)	2.0 ± 0.30	7.8 ± 0.34	1.4 ± 0.28	3.5 ± 0.24	8.0 ± 0.42
Bilateral (n = 8)	1.9 ± 0.73	9.0 ± 1.48	1.8 ± 0.54	3.2 ± 0.63	9.2 ± 1.86
Unilateral (n = 42)	2.0 ± 0.32	7.6 ± 0.34	1.4 ± 0.30	3.7 ± 0.25	8.1 ± 0.43
Canal intact (n = 17)	1.8 ± 0.54	6.1 ± 0.48[a]	1.0 ± 0.44	2.9 ± 0.42[c]	6.6 ± 0.56[e]
Canal deteriorated (n = 25)	2.2 ± 0.40	8.6 ± 0.34[b]	1.6 ± 0.41	4.2 ± 0.26[d]	9.0 ± 0.54[f]

Data are mean ± SD.

$p < .05$ by *t*-test for a vs b, c vs d, and e vs f.

RESULTS

Fifty patients underwent EDSS and were observed for 6 to 52 months. The results of EDSS were excellent, as described previously.[23] In this article, we put the long-term results of our surgery aside to assess them in later communications.

Severity of Postoperative Vestibular Symptoms. The subjective vestibular sensations and objective ENG findings of all 50 patients during the period of convalescence are listed in the Table. The average postoperative durations of static and motion-evoked vestibular sensations of all 50 patients were 2.0 and 7.8 days, respectively. Those of spontaneous, positional, and positioning nystagmus observed with ENG were 1.4, 3.5, and 8.0 days, respectively. As for the direction of nystagmus, of the 18 of 50 cases (36%) with spontaneous nystagmus after surgery, 7 cases changed from the operated side to the opposite side, 10 cases were observed only to the nonoperated side, and 1 case showed a couple of changes in direction. Of the 40 of 50 cases (80%) with positional nystagmus after surgery, nystagmus was directed to the nonoperated side in 33 cases when the head was positioned down toward the operated side.

Relationship Between Background Data and Severity of Postoperative Vestibular Symptoms. We statistically examined the data of all of the background factors mentioned in Materials and Methods that possibly influenced the postoperative course of vestibular symptoms. Patients with more frequent vertigo before surgery had more persistent spontaneous nystagmus after EDSS than those with less frequent preoperative vertigo ($p < .05$; Fig 1). In cases with a long history of Meniere's disease, postoperative static vestibular sensation ($p < .005$; Fig 2) and positional nystagmus ($p < .05$; data not shown) lasted significantly longer than in cases with a short history. However, there was no significant relationship between the other background factors and the postoperative course of vestibular symptoms (data not shown).

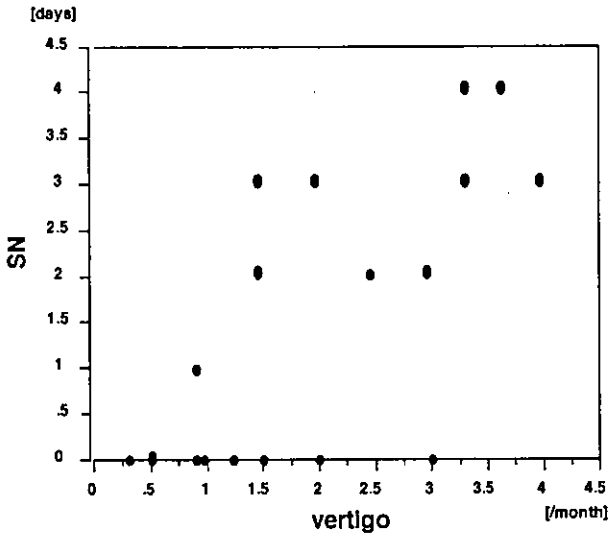

Fig 1. Postoperative duration in days of spontaneous nystagmus (SN; vertical axis) and frequency of definitive spells of vertigo for 6 months before operation (per month; horizontal axis). Patients with frequent preoperative vertigo had more persistent spontaneous nystagmus after surgery than those with less-frequent vertigo (Pearson test; $p < .05$).

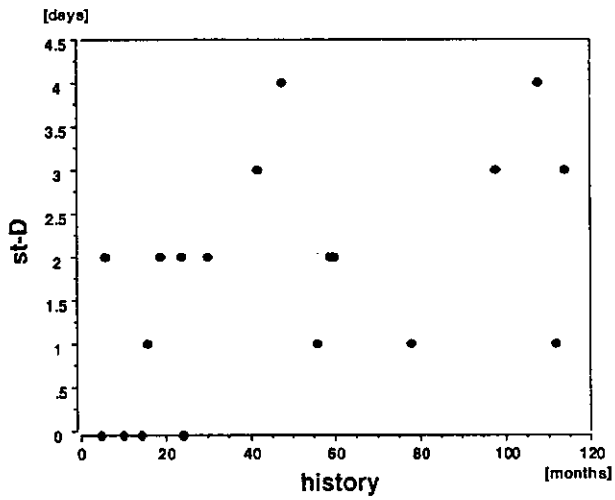

Fig 2. Postoperative duration (in days) of static vestibular sensation (static dizziness [st-D]; vertical axis) and preoperative duration (in months) of Meniere's disease (history; horizontal axis). In cases with long history of Meniere's disease, postoperative static vestibular sensation lasted significantly longer than in cases with short history of disease (Pearson test; $p < .05$).

Severity of Postoperative Vestibular Symptoms in Special Cases of Meniere's Disease. We compared the severity of postoperative vestibular symptoms between bilaterally (n = 8) and unilaterally affected cases (n = 42) and between unilateral cases with intact (n = 17) and deteriorated canals (n = 25; see Table). There was no significant difference in the decay profiles of any vestibular symptoms after EDSS between bilateral and unilateral cases. Postoperative motion-evoked vestibular sensation ($p < .05$) and positional ($p < .05$) and positioning nystagmus ($p < .05$) disappeared significantly earlier in unilateral cases with intact canals than in those with deteriorated canals.

DISCUSSION

In the present study, subjective and objective vestibular symptoms during the period of convalescence after EDSS were eliminated around 1 week after surgery. It is a matter of course that patients with frequent preoperative vertigo (factor 3) had more persistent spontaneous nystagmus after operation because the preoperative attacks could deteriorate vestibular peripheral function on the affected side in advance of surgery. In cases with a long history of Meniere's disease (factor 2), postoperative static vestibular sensation and positional nystagmus lasted significantly longer than in cases with a short history. The longer the duration of Meniere's disease, the worse the preoperative hearing levels and the CP scores,[2,3] so it is suggested that the postoperative vestibular symptoms lasted longer because of postoperative fragility in inner ears with long-standing disease. These symptoms were at most transient, and the inner ear functions were supposed to be improved over time.[23] However, patients with Meniere's disease have a tendency to be quite anxious about their dizzy feelings.[26] Therefore, the surgeon should remember to let them know in advance of their treatment how vestibular symptoms persist during the period of convalescence. There was no significant relationship between the other background factors, including the age of patients (factor 1), and the postoperative course of the vestibular symptoms. These results indicate that EDSS could be considered a less-invasive operation and be recommended even for elderly patients.

As for the direction of nystagmus after EDSS, 7 of the 18 cases of spontaneous nystagmus changed from the operated side to the opposite side and 10 cases had nystagmus only to the nonoperated side. We assume that the cases with nystagmus only to the nonoperated side did not show any nystagmus directed to the operated side because of the influence of anesthesia after the operation. Therefore, 18 of the 50 cases had spontaneous nystagmus after the operation; most had nystagmus that was directed to the operated side at first, was then directed to the nonoperated side, and finally disappeared. Because the postoperative CP scores were improved compared to the preoperative ones,[23] it is suggested that these courses of postoperative nystagmus could have been induced by recovery from the transiently irritated status of the vestibular periphery after EDSS.

The present study never revealed any significant differences in the decay profiles of EDSS-induced vestibular symptoms between bilaterally and unilaterally affected cases. This result indicates that EDSS could be as safe a treatment for bilateral Meniere's disease as for unilateral disease. In unilateral cases with intact canals, post-EDSS motion-evoked vestibular sensation and positional and positioning nystagmus disappeared significantly earlier than in those with deteriorated canals. This result indicates that EDSS could keep the vestibular peripheral function of patients with unilateral Meniere's disease and intact canals quite stable after surgery. On the other hand, the destructive operations, such as vestibular neurectomy[7,8] and intratympanic gentamicin treatment,[12-15] are considered to be quite effective in completely suppressing the definitive vertigo attacks that originate from Meniere's disease. However, these operations often cause treatment-induced dysequilibrium. As for vestibular neurectomy, Kubo et al[19] reported that spontaneous nystagmus and postural ataxia in the open-eyes condition resolved within about 2 weeks after neurectomy, whereas those in the closed-eyes condition lasted more than 3 weeks after surgery. Furthermore, it was revealed that postoperative compensation after vestibular neurectomy in bilateral cases was better when postoperative vestibular functions were preserved as much as possible.[17] As for intratympanic gentamicin therapy, the Helsinki University group reported that postoperative postural control became rather unstable for approximately 1 month as compared with postural control before surgery.[13] Furthermore, it was pointed out that there was a possibility of long-lasting unstable status, such as the jumbling phenomenon, in bilateral cases.[18] The findings of the present study suggest that these destructive therapies possibly cause more severe treatment-induced dysequilibrium than EDSS, especially in patients with bilateral Meniere's disease. Therefore, EDSS could be recommended as the initial less-invasive surgical treatment for intractable Meniere's disease, especially in unilateral cases with intact canals and in bilateral cases.

As mentioned above, the vestibular symptoms that resulted from direct invasion by EDSS were considered to be slighter than those caused by vestibular neurectomy or gentamicin treatment and were almost

the same as those caused by traditional endolymphatic sac surgery.[6] On the basis of the postoperative durations of vestibular symptoms found in the present study, we can easily plan patients' care and medication.

CONCLUSIONS

We have treated 50 patients with intractable Meniere's disease with EDSS, including cases from our previous preliminary report.[24] We observed subjective and objective vestibular symptoms with ENG during the period after surgery until patients returned to daily life.

1. The average postoperative durations of subjective static and motion-evoked vestibular sensations were 2.0 and 7.8 days, respectively. Those of spontaneous, positional, and positioning nystagmus observed with ENG were 1.4, 3.5, and 8.0 days, respectively.

2. There was no significant difference in the decay profiles of any postoperative vestibular symptoms between bilaterally (n = 8) and unilaterally affected cases (n = 42). In unilateral cases with no CP (n = 17), postoperative motion-evoked vestibular sensation, positional, and positioning nystagmus disappeared significantly earlier than in those with CP (n = 25).

3. We believe that EDSS could be recommended as an initial, less-invasive surgical treatment for intractable Meniere's disease, especially in unilateral cases with intact canals and in bilateral cases.

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Microarray analysis of gene expression in the rat vestibular nucleus complex following unilateral vestibular deafferentation

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Abstract

To investigate the molecular background of vestibular compensation, a model of lesion-induced plasticity, we used a microarray analysis to examine genes that show asymmetrical expression between the bilateral vestibular nucleus complexes (VNCs) 6 h following unilateral vestibular deafferentation (UVD). Asymmetrical gene expression was then validated by a real-time quantitative PCR. Among the 88 genes for which the ipsilateral (ipsi) : contralateral (contra) was > 1.35, the number of known genes was 33 (38%), and the number of expressed sequence tag (EST) sequences was 55 (62%). Among the 130 genes for which the contra : ipsi was > 1.35, the number of known genes was 55 (42%), and the number of EST sequences was 75 (58%). Changes in some of the genes were consistent with previous studies; however, we found several new genes which could be functionally related to the molecular basis of the electrophysiological asymmetry between the VNCs following UVD. Ipsi > contra genes included the $GABA_A$ receptor rho subunit, regulatory proteins of G protein signaling, calcium signaling related molecules such as the voltage-dependent calcium channel α2/δ subunit 1, calcineurin subunit Aβ and Ca^{2+} pump. Contra > ipsi genes included the neuronal high affinity glutamate transporter, 5-hydroxytryptamine receptor 1D, *mitogen-activated protein kinase 12* and ubiquitin carboxy-terminal hydrolase L1.

Keywords: gene expression, labyrinthectomy, microarray, mRNA, plasticity, vestibular compensation.

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Following unilateral vestibular deafferentation (UVD), many of the ocular motor and postural symptoms that persist in the absence of head movement, such as spontaneous ocular nystagmus and head tilt (i.e. 'static symptoms'), gradually abate over time in a process known as 'vestibular compensation'. In contrast, many of the changes that occur in the amplitude and timing of the vestibular reflexes in response to head movement, such as the reduced gain of the vestibulo-ocular reflexes (i.e. 'dynamic symptoms'), persist and appear to be permanent (for reviews, see Smith and Curthoys 1989; Curthoys and Halmagyi 1995; Dieringer 1995). The generation of the vestibular syndrome following UVD is related directly to the asymmetry in spontaneous resting activity between the brainstem vestibular nucleus complex (VNC) on the side of the lesion, and the VNC contralateral to it. Immediately after the UVD, resting activity in the ipsilateral (ipsi) VNC is largely abolished, while in the contralateral (contra) VNC it is markedly increased. The development of vestibular compensation of the static symptoms appears to be correlated with a partial recovery of spontaneous resting activity in the ipsi-VNC, thereby reducing the asymmetry between the bilateral VNCs (Darlington and Smith 2000). In terms of understanding the molecular basis of the symptoms of UVD and their compensation, it follows that the asymmetry in spontaneous resting activity that exists shortly following the UVD is a critical determinant of the severity of the vestibular syndrome and that understanding the changes in gene expression in the VNC that correlate with this

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Abbreviations used : AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; Contra, ontralateral; CREB, cAMP responsive element binding protein; CREM, cAMP responsive element modulator; EST, expressed sequence tag; GAD, glutamic acid decarboxylase; 5-HT, 5-hydroxytryptamine; Ipsi, ipsilateral; NFAT, nuclear factor of activated T-cells; RGS, regulators of G-protein signaling; UVD, unilateral vestibular deafferentation; VNC, vestibular nucleus complex.

asymmetry is important. Many studies have reported asymmetrical patterns of gene expression between the ipsi- and contra-VNCs following UVD (e.g. in expression of c-*fos*, GABA receptor, glutamate receptor, pro-opiomelanocortin receptor mRNA; Kitahara *et al.*; Darlington and Smith 2000). However, all of these studies have investigated only a small number of genes at one time. The development of gene microarray technology offers the possibility of investigating changes in the expression of thousands of genes simultaneously. In the present study, we used a microarray analysis to examine genes which show asymmetrical expression between the bilateral VNCs following UVD. This technique provided a powerful tool to detect changes in transcript expression in more than 10 000 samples in parallel. In some of the genes, asymmetrical expression detected by microarray analysis was confirmed by a real-time quantitative RT-PCR method.

Experimental procedures

Animals and unilateral vestibular deafferentation

All animal experiments were approved by the Animal Care Committee of Osaka University Medical School. Eight male Wistar strain rats weighing ≈180 g were used. Animals were anesthetised with pentobarbital (40 mg/kg, i.p.) and underwent a right UVD, using a retro-auricular approach. Local anesthesia with lidocaine was also used in the wound margin. After removal of the tympanic membrane, malleus and incus, the vestibule just above the ampullae of the horizontal and anterior semicircular canals was drilled out. After aspiration of labyrinthine fluids and the membraneous labyrinth from the drilled vestibule and the ventral portion of the oval window, the labyrinth was rinsed with 0.1 mL of absolute ethanol, and perfused through the ventral portion of the oval window and the drilled vestibule. As shown in Fig. 1, histological studies confirmed that the labyrinth was completely destroyed and that the lesion did not extend to the adjacent region such as ganglion cells.

Six hours following a right UVD, the animals were decapitated under ether anaesthesia and the brains were removed. The postoperative times were chosen on the basis of previous data on behavioural compensation in rats: 6 h post-UVD represents the acute, uncompensated stage when spontaneous nystagmus is vigorous and head tilt is severe (Kitahara *et al.* 1997; Horii *et al.* 2001).

Dissection of tissues and RNA extraction

Horizontal brainstem slices including the VNC (1.5 mm thickness) were taken just caudal from the abducens nucleus. Then, the bilateral VNCs were dissected separately under microscopic guidance using a sharp blade. The dissections were performed symmetrically using the detailed procedures we have described previously (Horii *et al.* 2001; Horii *et al.* 2002). All of these procedures were performed on a chilled plate in order to prevent possible RNA degradation. Total RNA was extracted using an RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The quality of extracted total RNA was confirmed with an Agilent Technologies 2100 Bioanalyzer-Bio Sizing (Agilent Technologies, Palo Alto, CA, USA). RNA samples

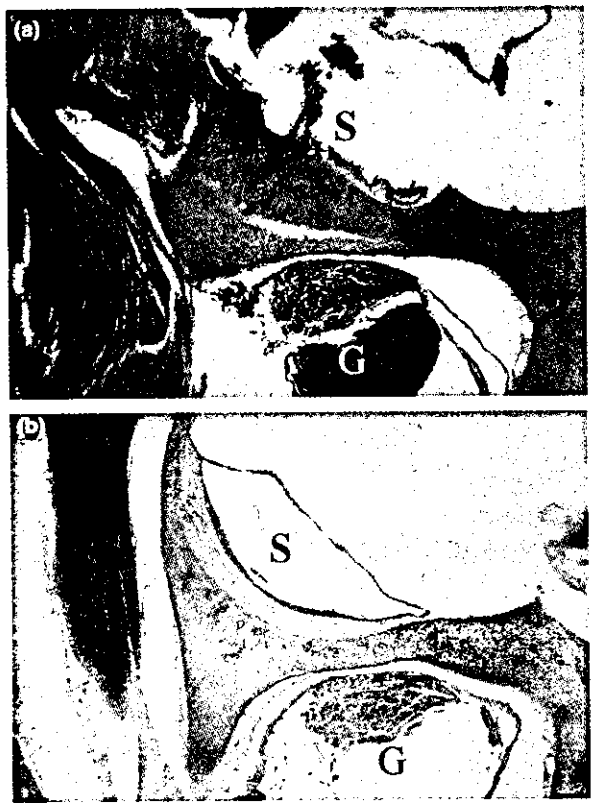

Fig.1 Microphotograph of inner ear of the UVD (a) and the control (b) animals. Saccular macula (S) of the UVD animal (a) was completely destroyed, however, adjacent ganglion cells (G) were preserved. Intact saccular macula (S) and ganglion cells (G) of the control animal (b) were also shown.

from eight ipsi (right) VNCs and eight contra (left) VNCs were pooled and forwarded to microarrays separately.

Target preparation and array hybridization

Precise methods for microarrays have been described elsewhere (Ramakrishnan *et al.* 2002). Experiments were conducted according to the user guide for CodeLink Expression Bioarrays (Amersham Biosciences, Piscataway, NJ, USA). In essence, single-stranded cDNA was prepared from 10 μg of total RNA using a T7-(dT)24-oligonucleotide primer and reverse transcriptase (200 U/μL). After double-stranded cDNA synthesis with DNA polymerase I (10 U/μL) and RNase H (2 U/μL), the cDNA served as the template in an *in vitro* transcription reaction to produce the target cRNA. The *in vitro* transcription was performed in the presence of biotinylated nucleotides to label the target cRNA. A set of bacterial control mRNAs was included in the cDNA synthesis kit as controls for the cDNA synthesis and *in vitro* transcription reactions. Each step of the procedure can be monitored using these control mRNAs.

The biotin-labeled cRNA was fragmented randomly by incubating 10 μg of the sample in the presence of magnesium for 20 min at 94°C. Ten micrograms of fragmented target cRNA was used for hybridization of a Uniset Rat CodeLink Bioarray chip, containing 10, 012 probes including 96 positive control genes and 250 negative control genes (Amersham). Information about these genes is

available through the following URL (http://www5.amershambiosciences.com/aptrix/upp01077.nsf/Content/codelink_literature).
These microarrays were hybridized, washed, and processed using a direct detection method of the biotin-labeled transcripts by Streptavidin–Alexa647 conjugates. Slides were scanned using CODELINK SCANNING Software (Motorola Life Sciences, Pasadena, CA, USA), and images for each slide were quantitated using the CODELINK EXPRESSION ANALYSIS Software (Motorola Life Sciences). Signal intensities for each spot were calculated by summation of the pixel intensities for each spot, then the local background (based on the median pixel intensity of the area surrounding each spot) was subtracted. Whole array data normalization was performed independently for each slide by dividing each spot's intensity (after background subtraction) by the median signal intensity of all test probes.

Real-time PCR

In three of the genes whose asymmetrical expression was detected by microarray analysis, these results were validated by a real-time quantitative RT-PCR method. RNA samples used for this validation experiment were exactly the same as those used for microarray analysis. The detailed procedures for the real-time quantitative PCR method have been published previously (Horii *et al.* 2001; Horii *et al.* 2002; Horii *et al.* 2003). Briefly, PCR was carried out with TaqMan® Universal PCR Master Mix (Perkin Elmer, Foster City, CA, USA). Each target molecule was coamplified with primers and the TaqMan® probe for glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in the same PCR tube. The final concentration of each oligonucleotide in the PCR reaction was as follows: GAPDH primer, 200 nM; primer for target molecule, 400 nM; TaqMan® probe for GAPDH and target molecule, 200 nM each. One microliter of cDNA was forwarded to PCR and the total reaction volume of PCR was 20 μL. Thermal cycling was initiated with incubation at 50°C for 2 min and 95°C for 10 min. After this initial step, 40 cycles of PCR were performed. Each PCR cycle consisted of heating at 95°C for 15 s for melting and 60°C for 1 min for annealing and extension.

The ABI7700 model software constructed amplification plots from the fluorescent emission data collected during the PCR amplification. CT (cycle of threshold) values corresponding to the number of PCR cycles at which the fluorescence emission monitored in real time exceeded a threshold limit (10× the standard deviation of the baseline intensity) were measured. The PCR assay for unknown samples was performed simultaneously with standard samples (i.e. VNC of sham-operated animals) and negative control samples (non-RT samples) in the same plate. The standard cDNA was diluted in four steps from 2× to 2^4×. A standard curve plotting CT values against input quantity was constructed for both GAPDH and the target molecule in every PCR assay. The relative concentration of GAPDH and the target molecule of unknown samples was calculated from this standard curve, and the ratio of the relative concentration of the target molecule to GAPDH (target molecule/GAPDH) of unknown samples was calculated. This ratio represented the relative expression of the target molecule of the unknown sample compared to the standard sample, which had been normalized by GAPDH expression. Possible variability in the initial amount of total RNA in each sample was controlled by this calibration procedure in which the target molecule was coamplified with an endogenously expressed GAPDH as an internal standard in the same PCR tube. Finally, the ratio (right : left) of gene expression of each target molecule was calculated. Sequences and fluorescent dyes of PCR primers and TaqMan® probes specific for the target molecules (calcineurin subunit Aβ, RGS9, striatin) and GAPDH were as follows. Calcineurin subunit Aβ: forward primer 5′-TTCCCTGAACACCGCACATA-3′, reverse primer 5′-CGCTGGTCACTGGGCACTAT-3′, probe 5′-CACTGAGAACCACGGGACTGGCAAC-3′; RGS9, forward primer 5′-CACCAAGAGAAGCTCCACCC-3′, reverse primer 5′-AGAATGACAGGGCTTGGGC-3′, probe 5′-CCATTTATGAGACGCCACCTACGCTCC-3′; Striatin, forward primer 5′-CCATCTGTTGGCTCCCCTT-3′, reverse primer 5′-TCTGCCCTACTGATGTCCTGC-3′, probe 5′-AGACCCAGCAGCTCCAGGCTTCCT-3′.

Results

Among the many genes investigated, we present here only genes for which the right (ipsi)–left (contra) differences in the signal intensity (right VNC : left VNC or left VNC : rightVNC) was > 1.35. This ratio was chosen because of its use in a previous microarray study (Bezchlibnyk *et al.* 2001). The number of genes, which showed a signal intensity ratio of > 1.35 for ipsi : contra or contra : right was 88 and 130, respectively. Among the 88 genes for which the ipsi : contra was > 1.35, the number of known genes was 33 (38%), and the number of expressed sequence tag (EST) sequences was 55 (62%). In the 130 genes for which the contra : ipsi was > 1.35, the number of known genes was 55 (42%), and the number of EST sequences was 75 (58%). Genes, accession numbers (NCBI) and ratio (ipsi : contra or contra : ipsi) by functional categories are shown in Tables 1 and 2, respectively.

Among these genes, we chose *calcineurin subunit Aβ*, *RGS9* and *striatin* as representatives and performed validation experiments using a real-time PCR. ipsi : contra of gene expression for *calcineurin subunit Aβ*, *RGS9* and *striatin* obtained by microarray analysis was 1.83, 1.44 and 1.42, respectively (Table 1). Real-time PCR studies demonstrated that the ipsi : contra of the same genes were 1.17, 1.53 and 1.19, respectively (Table 3). It is indicated that microarray data were closely correlated with real-time PCR results.

Discussion

We could detect several genes showing ipsi : contra differences in expression in the VNC at 6 h following UVD. Ramakrishnan *et al.* (2002) validated the differential expression ratios obtained with the CodeLink microarray system, as used in the present study, against those obtained with quantitative reverse transcription-PCR assays for 54 genes. It was concluded that there was a good correlation (correlation coefficient of 0.76) in the changes reported by both systems. We also confirmed the present microarray data by a real-time

Table 1 Genes, accession numbers (NCBI) and ratio (ipsi : contra) by functional categories

Function	Accession number	Gene	Ipsilateral : contalateral
Signal transduction and regulation	NM_017292	*GABA-A receptor, subunit Rho 2*	1.36
	NM_019344	*Regulator of G-protein signaling 8 (RGS8)*	1.37
	NM_017031	*Phosphodiesterase 4B, cAMP-specific [dunce (Drosophila)-homolog phosphodiesterase e4] (PDE4B)*	1.38
	NM_017129	*Cardiotrophin 1 (CTF1)*	1.4
	NM_019148	*Striatin (STRN)*	1.42
	NM_019224	*Regulator of G-protein signaling 9 (RGS9)*	1.44
	NM_134417	*Inositol polyphosphate multikinase (IPMK)*	1.57
	NM_030871	3'-RACE clone 8 *phosphodiesterase 1A (PDE1A)*	1.65
	NM_017042	*Calcineurin Subunit Aβ (PPP3CB)*	1.83
	NM_133619	*Glycoprotein hormone α2 (GPHA2)*	1.87
Membrane transport	NM_012919	*Calcium channel, voltage-dependent, alpha2/delta subunit 1 (CACNA2D1)*	1.38
	NM_053424	*Solute carrier family 4, sodium bicarbonate cotransporter, member 4 (SLC4A4)*	1.48
	NM_053311	*ATPase, Ca^{2+} transporting, plasma membrane 1 (ATP2B1)*	1.6
Protein modification	NM_031766	*Carboxypeptidase Z (CPZ)*	1.35
	AF053094	*Pachytene spermatocytes methoxy acetic acid induced gene*	1.47
	NM_031081	*3-Phosphoinositide dependent protein kinase-1 (PDPK1)*	1.5
Nuclei acid synthesis and modification	U34843	*Cell cycle progression related D123*	1.37
	NM_017248	*Heterogeneous nuclear ribonucleoprotein A1 (HNRPA1)*	1.42
	NM_080910	*Phosphoribosylaminoimidazole carboxylase, phosphoribosylaminoribosylaminoimidazole succinocarboxamide synthetase (PAICS)*	1.42
	NM_013135	*RAS P21 protein activator (RASA1)*	1.44
	NM_019149	*Matrin 3 (MATR3)*	1.66
	U17013	*Transcription factor OCT1 (OCT1)*	2.12
Adhesion and molecular recognition	NM_019621	*Discs, large homolog 4 (Drosophila) (DLGH4)*	1.42
	NM_013045	*Tenascin-R (restrictin, janusin, J1-160/180) (TNR)*	1.52
	U53475	*GTPase RAB8B (RAB8B)*	1.57
Electron transfer	NM_053850	*Biliverdin reductase A (BLVRA)*	1.37
Membrane	NM_053438	*Zinc finger protein 103 (ZFP103)*	1.37
	NM_053534	*Transmembrane domain protein regulated in adipocytes 40 kDa (TPRA40*-pending*)*	1.41
	AJ277881	*Conjugate export pump protein*	1.44
Cytoskeleton	NM_053567	*Formiminotransferase cyclodeaminase (FTCD)*	1.45
	NM_130740	*Protein kinase C and casein kinase substrate in neurons 2 (PACSIN2)*	2.06
Others	U96490	*Liver mRNA*	1.42
	NM_053946	*Implantation-associated protein (IAG2)*	1.52

PCR. Therefore, the data obtained by the present microarray analysis are reliable. As we did not compare gene expression with sham-controls in this first study, it is impossible to distinguish up-regulation (down-regulation) in the ipsi-VNC from down-regulation (up-regulation) in the contra-VNC. Furthermore, changes in mRNA levels are, of course, not necessarily indicative of changes in protein expression and function. Nonetheless, the asymmetry in electrophysiological activity and molecular expression between the ipsi- and contra-VNCs is the driving force behind the severity of the vestibular syndrome following UVD, and this study is the first to demonstrate changes in gene expression following UVD using a microarray analysis. In contrast, the present microarray experiments also confirmed many of the negative data reported by the previous *in situ* hybridization studies (de Waele *et al.* 1994; Rabbath *et al.* 2002): the present study demonstrated no ipsi : contra differences in gene expression for *NMDAR1*, *GluR2* and *GluR4* subunit/subtypes of glutamate receptors (data not shown).

Ipsilateral : contralateral > 1.35 genes

Among 33 known genes which showed right/left ratios > 1.35, 10 genes were classified into the signal transduction and regulation categories. The $GABA_A$ receptor Rho subunit was detected in accordance with a previous study in which other $GABA_A$ subunits (e.g. α1) were up-regulated in the ipsi-VNC following UVD (Horii *et al.* 2003). Two regulators of G-protein signaling (RGS8 and RGS9) were also detected.

Table 2. Genes, accession numbers (NCBI) and ratio (contra : ipsi) by functional categories

Function	Accession number	Gene	Contalateral : ipsilateral
Signal transduction and regulation	NM_012656	*Secreted acidic cystein-rich glycoprotein (osteonectin) (SPARC)*	1.35
	NM_017007	*Glutamate decarboxylase 1* (brain) (*GAD1*)	1.35
	NM_021746	*Mitogen-activated protein kinase 12 (MAPK12)*	1.39
	NM_019196	*Multiple PDZ domain protein (MPDZ)*	1.4
	NM_019339	*Regulator of G-protein signaling 12 (RGS12)*	1.4
	AF065387	γ-Glutamyl carboxylase (GGCX)	1.41
	D63772	*Neuronal high affinity glutamate transporter*	1.41
	NM_013086	*cAMP responsive element modulator (CREM)*	1.42
	AF271235	*Differentation-associated Na-dependent inorganic phosphate cotransporter (DNPI)*	1.43
	AF115249	*Sphingosine 1-phosphate receptor (EDG8)*	1.44
	L16532	(Clone PCNPII) *2′,3′*-cyclic nucleotide 3′-phosphodiesterase (CNPII)	1.44
	NM_019380	*Stromal cell derived factor receptor 1*	1.47
	AF181259	*Leucine zipper protein 1 (LUZP1)*	1.49
	NM_020073	*Parathyroid hormone receptor (Loc56813)*	1.78
	NM_012852	*5-HT$_{1D}$ receptor (HTR1D)*	1.79
	NM_013130	*MAD (mothers against decapentaplegic, Drosophila) homolog 1 (MADH1)*	1.81
Membrane transport	M58758	ATPase, *H$^+$ transporting, lysosomal (vacuolar proton pump) noncatalytic accessory protein 1* (110/160 kDa) (*ATP6N1*)	1.37
	NM_012505	ATPase, *Na$^+$/K$^+$ transporting, alpha 2 polypeptide* (*ATP1A2*)	1.51
Protein modification	AB012759	*Prolyl endopeptidase (PREP)*	1.37
	AF012891	*Secreted frizzled-related protein 4 (SFRP4)*	1.37
	AF220455	*Regulator of steroidogenic factor-1*	1.39
	J03190	*Aminolevulinic acid synthase 1 (ALAS1)*	1.41
	NM_012500	*N-Acylaminoacyl-peptide hydrolase (APEH)*	1.41
	NM_017237	*Ubiquitin carboxy-terminal hydrolase L1 (UCHL1)*	1.41
	AB012139	*Procollagen c-proteinase 3*	1.52
	AF227909	*Calpain 10* (*CAPN10*)	1.53
	NM_013185	*Hemopoietic cell tyrosine kinase (HCK)*	1.56
	NM_012939	*Cathepsin H (CTSH)*	1.63
	L12382	*ADP-ribosylation factor 3 (ARF3)*	1.65
	AB026288	*Pharbin*	1.75
	AF190121	*Growth Factor Receptor Binding Protein GRB7 (GRB7)*	2.58
Nuclei acid synthesis and modification	AB047556	*Transcription factor Usf2 (Usf2)*	1.38
	AF000578	*CDC5* (*cell division cycle 5, Schizosaccharomyces pombe,* homolog)-LIKE (*CDC5L*)	1.4
	AF036255	*Ring finger protein 22 (RNF22)*	1.42
	NM_021662	*DNA polymerase delta, catalytic subunit (POLD1)*	1.43
	AF110195	*Importin 13 (IMP13)*	1.53
Adhesion and molecular recognition	AB006461	*Neurochondrin (NCDN-pending)*	1.43
Cytoskeleton	NM_021997	*Cytoplasmic linker 2 (CYLN2)*	1.39
	AB032827	*Erythrocyte membrane protein band 4.1-like 3 (EPB41L3)*	1.4
	NM_019234	*Dynein, cytoplasmic, intermediate chain 1 (DNCIC1)*	1.43
	NM_017029	*Neurofilament protein, middle polypeptide* (*NEFM*)	1.49
Metabolism	AB013732	*UDP-glucose dehydrogeanse (UGDH)*	1.38
	X13295	*lipocalin 2 (lcn2)*	1.41
	AB021980	*Delta-6 fatty acid desaturase (FADS2)*	1.42
	AF047707	*UDP-glucose:ceramide glycosyltransferase*	1.45
	AF061266	*TRP1 beta variant*	1.52
	D50580	*Carboxylesterase*	1.97

Table 2. (Continued)

Function	Accession number	Gene	Contalateral : ipsilateral
Others	U93692	*Preimplantation protein 2 (PREI2)*	1.36
	NM_019213	*Jumping translocation breakpoint (JTB)*	1.38
	AF304429	*Brain protein 44-like (BRP44L)*	1.4
	NM_019290	*B-cell translocation gene 3 (BTG3)*	1.4
	NM_019277	*SEC15 homolog (Saccharomyces cerevisiae) (SEC15)*	1.51
	AJ293617	*Melanoma antigen, family D, 2 (MAGED2)*	1.55
	AF092207	Unknown mRNA	1.65
	U54807	*RAB3C*, member ras oncogene family *(RAB3C)*	1.92

Table 3. Validation of microarray data by real-time PCR methods

Gene	Microarray (Ipsi : contra)	Real-time PCR (Ipsi : contra)
Calcineurin subunit Aβ	1.83	1.17
RGS9	1.44	1.53
Striatin	1.42	1.19

RGS8 was shown to regulate G-protein coupled-receptors as a GTPase-activating protein which drives G proteins into their inactive GDP-bound form (Saitoh *et al.* 1997). RGS9 was reported to interact with dopamine D2 receptors and have a role in compensatory adaptation to psychostimulant responsiveness (Rahman *et al.* 2003). VNC neurons have several G-protein-coupled metabotropic receptors (see Smith and Darlington 1996 for a review), and these regulators of G-protein signaling (RGS8 and RGS9) might interact with these receptors and bidirectionally affect their expression and function following UVD. cAMP-specific phosphodiesterase 4B is a homolog of the *dunce (dnc)* learning and memory gene of *Drosophila*, which is essential for olfactory learning and female fertility in *Drosophila* but has also shown expression in the mammalian CNS (Bolger *et al.* 1994). Cardiotrophin 1 is primarily released from the heart in response to hypoxic stress and it protects cardiac myocytes from hypoxia-induced apoptosis (Craig *et al.* 2001). Regarding its central role, cardiotrophin 1 is known as a neurotrophic factor and it promotes the outgrowth of embryonic cranial motoneurons (Naeem *et al.* 2002). Striatin, isolated from synaptosomal proteins, is reported to interact with dendrites of excitatory synapses in the central nervous system in a CaM/Ca^{2+}-dependent manner (Castets *et al.* 1996). Gene expression of protein kinase C and casein kinase substrate in neurons was higher in the ipsi-VNC. Previous studies demonstrated changes in protein phosphorylation in the vestibular nucleus following UVD (see Darlington and Smith 2000 for a review). Taken together, this molecule may be one of the substrates for phosphorylation. Calcineurin is a Ca^{2+} and CaM-dependent protein phosphatase which influences a variety of proteins including ion channels (Ca^{2+} channels), neurotransmitter receptors (NMDA, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), $GABA_A$, inositol triphosphate (IP3)), enzymes (neuronal-nitric oxide synthase (nNOS), glutamic acid decarboxylase (GAD)), and transcription factors (cAMP responsive element binding protein (CREB), nuclear factor of activated T-cells c4 (NFATc4)) (Groth *et al.* 2003). As a result of the above cellular mechanisms, calcineurin is reported to have a role in learning/memory at the behavioural level (Groth *et al.* 2003).

Ca^{2+}-related molecules such as the voltage dependent α2/δ subunit of the Ca^{2+} channel and Ca^{2+} pump (Ca^{2+} transporting ATPase) were altered following UVD. Changes in other Ca^{2+} related- or dependent molecules such as calcineurin, RGS8, RGS9, striatin were also detected. It has been reported that Ca^{2+} channel blockers can reduce the symptoms of UVD (see Darlington and Smith 2000 for a review). Taken together, molecules involved in the regulation of intracellular Ca^{2+} concentrations might be important in the asymmetry in neuronal activity between the bilateral VNCs that occurs following UVD and during the development of vestibular compensation.

Contralateral : ipsilateral > 1.35 genes

Among 55 known genes which showed contra : ipsi > 1.35, 16 genes were classified into the signal transduction and regulation categories. The differentiation-associated Na^+-dependent inorganic phosphate cotransporter is now recognised to be a vesicular glutamate transporter (Hayashi *et al.* 2001); gene expression for this protein may change following UVD because of the large increase in glutamate released into the contra-VNC immediately after UVD (Inoue *et al.* 2003). Along the same lines, gene expression for the neuronal high affinity glutamate transporter (for a review, see Kanai and Hediger 2003) may increase contralaterally because of the asymmetry in glutamate release into the two VNCs. It is of interest that the expression of the *5-HT_{1D}* receptor gene changes in the VNC following UVD. Although

5-HT receptor expression in the VNC has not been studied in relation to the effects of UVD and the development of vestibular compensation, a number of 5-HT receptors have been found to contribute to normal VNC neuronal function (Smith and Darlington 1996). Among regulators of G-protein signaling genes, gene expression of *RGS8* and *RGS9* were higher in the ipsi-VNC (Table 1) and *RGS12* was lower in the contra-VNC following UVD (Table 2). VNC neurons have several G-protein-coupled metabotropic receptors (Smith and Darlington 1996), and these genes might interact with metabotropic receptors and affect their expression and function following UVD. The cAMP responsive element modulator (CREM), which forms complexes with cAMP responsive element binding protein (CREB), is an important part of the cAMP signaling system and may regulate the activity of cytokines such as interleukin-2 (Tenbrock *et al.* 2003). Leucine zipper protein 1 is a member of a family of proteins involved in modulating the interaction of transcription factors with leucine zipper binding domains (Zhao *et al.* 2003), of which two are the immediate early gene transcription factors, c-*fos* and c-*jun*. As the expression of both of these transcription factors in the VNC is associated with the effects of UVD, the change in *leucine zipper protein 1* gene expression may be related to changes in their regulation of late-response genes following vestibular damage. On the basis of previous data, it is not surprising that glutamic acid decarboxylase 1 (*GAD1*) gene expression changes in the VNC following UVD. However, our RT-PCR studies of *GAD65* and *GAD67* in the VNC at 6 h following UVD have indicated a decreased expression in the contra-VNC relative to the ipsilateral side (Horii *et al.* 2003). The reason for this apparent discrepancy is not clear at present.

Among 55 known genes which showed contra : ipsi > 1.35, 13 genes were classified into the protein modification categories. Pharbin, a novel inositol polyphosphate 5-phosphatase, induces dendritic appearance in fibroblasts (Asano *et al.* 1999). Neurochondrin/norbin is a cytoplasmic protein involved in the regulation of dendritic outgrowth (Mochizuki *et al.* 2003). It is conceivable that increased expression of these genes in the contra-VNC could be involved in synaptic plasticity initiated by the imbalance in resting activity between the two VNCs. Growth factor receptor binding protein GRB7 is one of a family of growth factor receptor binding proteins which can interact with the insulin receptor and insulin-like growth factor I receptor (Vecchione *et al.* 2003). The change in its gene expression in the VNC following UVD may be related to the role of growth factors in initiating plasticity following the lesion. Changes in the expression of the gene for 'regulator of steroidogenic factor-1' could play a similar role (Wehrenberg *et al.* 2001). Calpain 10, on the other hand, is a member of the calpain family of proteases, and may be involved in the regulation of insulin-stimulated glucose uptake (Paul *et al.* 2003).

Erythrocyte membrane protein band 4.1-like 3 is one of the band 4.1 superfamily which is expressed in brain tissue and may have a role in the regulation of the cytoskeleton (Takeuchi *et al.* 1994). The sphingosine-1-phosphate receptor is also known to be implicated in cytoskeletal remodelling (Ozaki *et al.* 2003). Neurofilament protein middle polypeptide has been shown to be a marker for white matter changes and changes in its expression following UVD may reflect alterations in myelination (Sjogren *et al.* 2001). The multiple PDZ domain protein, on the other hand, is involved in many aspects of cell signaling given the ubiquitous nature of PDZ domains (Jelen *et al.* 2003). Changes in its gene expression, and the expression of the *mitogen-activated protein kinase 12* gene, could reflect many different aspects of synaptic plasticity in the VNC, including neurite outgrowth (Park *et al.* 2003). Ubiquitin carboxy terminal hydrolase-L1 is a neuron-specific deubiquitinating enzyme, which is shown to be involved in ubiquitin expression after several stress stimuli (Harada *et al.* 2004). Expression of this gene in the contra-VNC following UVD may be related to a stress response in the VNC.

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Paroxetine, a Selective Serotonin Reuptake Inhibitor, Reduces Depressive Symptoms and Subjective Handicaps in Patients with Dizziness

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Objective and Study Design: When treating dizzy patients, the psychiatric aspect should be carefully addressed regardless of whether a well-defined organic disease is present. In this prospective study, we aimed to elucidate the role of paroxetine, a selective serotonin reuptake inhibitor, in the treatment of dizziness.

Setting and Patients: Forty-seven patients who complained of dizziness were treated with 20 mg of paroxetine per day. The depressive state of the patient was evaluated by the Zung Self-Rating Depression Scale (SDS). Treatment outcomes were measured with self-assessment of subjective handicaps in daily life using a dizziness and unsteadiness questionnaire. The questionnaire consisted of five factors related to emotional or bodily dysfunction that could be affected by dizziness. Changes in Self-Rating Depression Scale scores and subjective handicaps were assessed at 4 and 8 weeks after the start of paroxetine.

Results: In patients having well-defined organic diseases with high Self-Rating Depression Scale scores, paroxetine improved all five subjective handicap factors as well as Self-Rating Depression Scale scores. The decline in Self-Rating Depression Scale scores showed a significant correlation with improvement of subjective handicaps, which was related to emotional problems but not factors related to bodily dysfunction. Paroxetine was also effective for an improvement of factors related to emotional problems and Self-Rating Depression Scale scores in patients not having organic diseases but with high Self-Rating Depression Scale scores. In patients either with or without organic diseases with low Self-Rating Depression Scale scores, paroxetine had no effect on any subjective handicap factors and Self-Rating Depression Scale scores.

Conclusion: In the treatment of dizzy patients, paroxetine was effective at relieving subjective handicaps caused by dizziness, specifically, in patients with high Self-Rating Depression Scale scores. **Key Words:** Depression—Dizziness—Self-Rating Depression Scale—Serotonin—Serotonin selective reuptake inhibitor—Vertigo—Vestibular.

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Vertigo and dizziness mainly result from dysfunction of peripheral and/or central vestibular systems; however, psychiatric disorders such as depression and anxiety sometimes cause vertigo and dizziness (1). Alternatively, vestibular dysfunction may initiate development of psychiatric disorders such as depression and anxiety and preserve subjective vertigo and dizziness despite remission of or central compensation for the peripheral lesion (2). Therefore, in the treatment of dizzy patients, it is important to address the psychiatric status, regardless of whether they have a well-defined organic disease (3). If patients are diagnosed as having psychiatric disorders, it is necessary to treat not only vestibular dysfunction but also psychiatric disorders with adequate medication such as antidepressants, benzodiazepines, and/or psychotherapy.

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Recently, selective serotonin reuptake inhibitors (SSRIs) have been effectively used for the treatments of depression and anxiety (4). To date, only one report on the effects of SSRIs on dizziness with psychiatric disorders is available (5). In the current study, we aimed to elucidate the role of SSRIs in the treatment of dizziness in patients with or without depressive state. The depressive state of patients was evaluated by using the Zung Self-Rating Depression Scale (SDS) (6) in Japanese translation (7). Treatment outcomes were measured with the self-assessment of handicaps in daily life caused by vertigo and dizziness using a dizziness and unsteadiness questionnaire established by our department (8,9) as well as bedside and laboratory examinations by clinicians.

PATIENTS AND METHODS

Consecutive patients with complaints of dizziness and/or vertigo who visited the Department of Otolaryngology, Osaka University Medical School, were asked whether they agreed to enter the study. Before entering the study, informed consent regarding the purpose of this study and possible effects and

adverse effects of the drug was obtained from each patient. There were no patient selection criteria. This study was approved by the local committee of the department and performed in accordance with the Declaration of Helsinki.

Diagnosis, medical treatment, and measurement of treatment outcome

Otoneurologic examinations including smooth pursuit test, observation of nystagmus with an infrared charge-coupled device camera, a postural test, and pure-tone audiometry were performed. Neuroimaging such as computed tomography or magnetic resonance imaging/magnetic resonance angiography of the brain and other examinations including caloric test, glycerol test, and electrocochleography were also performed if clinically indicated. On the basis of these examinations and careful interviews of history, otoneurology specialists diagnosed patients according to the guidelines for the diagnosis of vertigo and dizziness established by the Japan Society for Equilibrium Research.

During the initial 2 weeks, patients were asked to take 10 mg of paroxetine per day. Then, during the after 6 weeks, they were asked to take 20 mg/d. Five milligrams of metoclopramide was also prescribed just in case nausea, an adverse effect of paroxetine, occurred. No other medicine was used. However, if a patient had already been prescribed other medicine, they were allowed to keep using it.

Subjective handicaps in daily life due to vertigo and dizziness were assessed using a dizziness and unsteadiness questionnaire created by our department (8,9). The questionnaire consisted of 14 items (Table 1). As the assessment, the answers to all the questions were scored 1 to 5 on a scale as follows: severe handicap = 5, significant handicap = 4, moderate handicap = 3, slight handicap = 2, and no handicap = 1 due to the symptom. Then, scores were collected according to a factor classification. Questions 1, 5, and 9 belong to Factor 1, disturbance of social activity; Questions 2, 6, and 10 belong to Factor 2, body motion precipitating dizziness; Questions 3, 7, and 11 belong to Factor 3, limitation of physical activity; Questions 4, 8, and 12 belong to Factor 4, emotional disturbance; and Questions 1, 12, and 13 belong to Factor 5, disturbance of interpersonal communications.

The depressive status of each patient was assessed by the SDS with Japanese translation (7). Before, 4 weeks after, and 8 weeks after the start of paroxetine, SDS scores, subjective handicaps assessed by the questionnaire, and clinicians' evaluations regarding abnormal findings were obtained. Clinicians' evaluations were defined as the assessment of only objective abnormal findings on otoneurologic examinations irrespective of the patients' complaints. Saccadic eye movement, gaze-evoked nystagmus, spontaneous nystagmus, positional nystagmus, head shaking nystagmus, and postural deviation were regarded as abnormal. Score 2 was given to patients with abnormal findings, whereas Score 1 was given to patients without abnormal findings.

Background of patients and classification of patients into Group I to Group IV

Of the 52 patients who entered the study, 4 patients could not keep taking the medicine because of nausea, and the data for 1 patient could not be retrieved for an unknown reason. We analyzed data from the other 47 patients (Table 2). Included were 16 patients with Ménière's disease, 3 with delayed endolymphatic hydrops, 6 with vestibular neuritis, 1 with unilateral peripheral vestibular dysfunction, 1 with bilateral peripheral vestibular dysfunction, 1 with benign paroxysmal positional vertigo, and 1 with posttrauma dizziness. No evident organic diseases were found in the other 18 patients.

TABLE 1. *The dizziness and unsteadiness questionnaire*

1. Do you refrain from going out or traveling for work or amusement due to dizziness or unsteadiness?
   (a) Always
   (b) Frequently
   (c) Sometimes
   (d) Rarely
   (e) No
   (f) No idea
2. Do you hate walking in dark places even around your home due to dizziness or unsteadiness?
   (a) Absolutely
   (b) Significantly
   (c) Moderately
   (d) Slightly
   (e) No
   (f) No idea
3. Do you hate going downstairs due to dizziness or unsteadiness?
4. Do you feel annoyed due to dizziness or unsteadiness?
5. Do you feel that you are not able to do your work either at home or at an office due to dizziness or unsteadiness?
6. Is the degree of dizziness or unsteadiness strengthened when you suddenly move your head (e.g., when looking back)?
7. Do you hate walking through narrow spaces (e.g., narrow sidewalk) due to dizziness or unsteadiness?
8. Do you feel that you have a physical handicap and are inferior to other persons due to dizziness or unsteadiness?
9. Are you unable to concentrate on something due to dizziness or unsteadiness?
10. Do you think it is too much trouble to read books or newspaper due to dizziness or unsteadiness? Or do you have some trouble in reading them?
11. Is the degree of dizziness or unsteadiness strengthened when you stand up from a chair?
12. Do you feel anxiety about yourself when you are in the presence of others due to dizziness or unsteadiness?
13. Do you refrain from meeting or going out with your family or friends due to dizziness or unsteadiness?
14. Do you have difficulties in your daily life due to dizziness or unsteadiness?

The SDS is a 20-item self-report measure of the symptoms of depression. Although the scores are not meant to offer strict diagnostic guidelines but rather denote depressive symptoms, the SDS has been shown to be valid in clinical use. Full scores are 80, and less than 48 is considered normal in the Japanese version (7). Therefore, we divided the patients into a high-SDS group (SDS $\geq$48) and a low-SDS group (SDS <48).

Each patient was placed into one of four groups depending on the presence of organic disease (positive or negative) and his or her SDS score (high or low): Group I, organic disease-positive with a high SDS score; Group II, organic disease-positive with a low SDS score; Group III, organic disease-negative with a high SDS score; and Group IV, organic disease-negative with a low SDS score. Table 2 shows patient background information including age, sex, months after onset, hearing level, caloric paresis, SDS scores, and diseases of each group. Except for hearing level in Group I and SDS scores in Groups I and III, there were no differences in the patients' background information between each group.

Statistical analysis

Differences in age, months after onset, hearing level, caloric paresis, SDS scores at premedication, and self-assessment of handicaps evaluated by the questionnaire between Groups I

TABLE 2. *Patient background information*

	Group I, organic disease-positive, high SDS score (n = 12)	Group II, organic disease-positive, low SDS score (n = 17)	Group III, organic disease-negative, high SDS score (n = 6)	Group IV, organic disease-negative, low SDS score (n = 12)
Mean age (yr) (SD)	54.4 (15.5)	53 (14.8)	36.0 (12.1)	51.9 (11.0)
Sex				
Male (%)	5 (42)	10 (59)	1 (20)	6 (50)
Female (%)	7 (58)	7 (41)	5 (80)	6 (50)
Months after onset (SD)	3.1 (3.4)	6.6 (7.8)	3.0 (2.3)	5.6 (8.4)
Hearing level (dB) (SD)	51 (30.3)[a]	34.5 (23.7)	17.9 (3.7)	20.0 (7.8)
Caloric paresis (%) (SD)	40.8 (30.0)	44.8 (36.3)	12.7 (12.1)	11.2 (6.6)
SDS score (SD)	57.4 (7.6)[b]	37.8 (6.9)	53.8 (5.4)[b]	37.3 (7.5)
Disease	8 MD, 3 VN, 1 DEH	8 MD, 3 VN, 2 DEH, 1 UVD, 1 BVD, 1 BPPV, 1 Trauma		

[a] $p < 0.01$ versus Group III.
[b] $p < 0.01$ versus Group II and Group IV.
MD, Ménière's disease; VN, vestibular neuritis; DEH, delayed endolymphatic hydrops; UVD, unilateral vestibular dysfunction; BVD, bilateral vestibular dysfunction; BPPV, benign paroxysmal positional vertigo; Trauma, posttrauma dizziness.

through IV were tested by analysis of variance (ANOVA) followed by the Bonferroni/Dunn post hoc test. Differences in self-assessment of subjective handicaps evaluated by the questionnaire, SDS scores, and clinicians' evaluation 4 weeks or 8 weeks after the start of the SSRI were compared with those of premedication value using the Wilcoxon signed ranks test. The correlation coefficient of declines in SDS score and each factor of self-assessment of subjective handicaps (Factors 1–5) after 8 weeks of medication was calculated and tested using Fisher's r to z (p values).

RESULTS

SDS score and subjective handicaps in daily life due to dizziness before paroxetine

Of the 47 patients in the current study, 18 patients (Groups I and III) showed a high SDS score (≥48) before paroxetine. As shown in Table 3, ANOVA followed up by the Bonferroni/Dunn post hoc test showed that all five subjective handicaps factors before medication in Group IV (organic disease-negative with a low SDS score) were significantly lower compared with Group I (organic disease-positive with a high SDS score) and that Factors 1, 2, and 4 were also lower in Group II (organic disease-positive with a low SDS score) compared with Group I.

TABLE 3. *Self-handicaps in daily life due to dizziness before serotonin selective reuptake inhibitors by group*

	Group I (n = 12)	Group II (n = 17)	Group III (n = 6)	Group IV (n = 12)
Factor 1	12.5 ± 2.5	9.1 ± 3.4[a]	12.5 ± 2.1	8.5 ± 4.3[a]
Factor 2	10.8 ± 1.8	7.5 ± 3.0[a]	9.0 ± 2.0	6.3 ± 1.7[a]
Factor 3	9.9 ± 2.7	7.4 ± 3.7	8.0 ± 2.0	5.6 ± 1.9[a]
Factor 4	11.1 ± 2.0	8.3 ± 3.7[a]	8.8 ± 4.2	4.8 ± 2.3[a]
Factor 5	12.4 ± 1.8	9.6 ± 4.2	11.3 ± 3.1	6.9 ± 3.3[a]
Clinicians' evaluation	1.6 ± 0.5	1.7 ± 0.5	1.0 ± 0.0	1.0 ± 0.0

[a] $p < 0.01$ versus Group I.
Factor 1, disturbance of social activity; Factor 2, body motion precipitating dizziness; Factor 3, limitation of physical activity; Factor 4, emotional disturbance; and Factor 5, disturbance of interpersonal communications.
Group I, organic disease-positive with a high SDS score; Group II, organic disease-positive with a low SDS score; Group III, organic disease-negative with a high SDS score; Group IV, organic disease-negative with a low SDS score.

Group I: organic disease-positive with a high SDS score

The diagnoses in this group were eight with Ménière's disease, three with vestibular neuritis, and one with delayed endolymphatic hydrops. All 12 patients showed a decrease in SDS scores 8 weeks after medication. As shown in Figure 1B, the SDS score of this group before medication was 57.4 ± 7.6 (mean ± SD) and that at 8 weeks after medication (44.8 ± 9.9) was normal (<48). This is a significant decrease (Wilcoxon signed ranks test, $p = 0.0033$). At 8 weeks after medication, all 12 patients showed recovery in Factors 1, 3, 4, and 5, whereas 11 of 12 patients showed recovery in Factor 2. As shown in Figure 1A, all five subjective handicaps factors evaluated by the questionnaire significantly improved after 8 weeks of medication (Wilcoxon signed ranks test: Factor 1, $p = 0.0033$; Factor 2, $p = 0.0076$; Factor 3, $p = 0.0129$; Factor 4, $p = 0.0033$; and Factor 5, $p = 0.0077$). Objective evaluation by clinicians did not show recovery (Fig. 1C). The decrease in the SDS score showed a significant correlation with improvement of Factor 1 (disturbance of social activity, $r = 0.803$), Factor 4 (emotional disturbance, $r = 0.694$), and Factor 5 (disturbance of interpersonal communications, $r = 0.764$), but not with Factor 2 (body motion precipitating dizziness) and Factor 3 (limitation of physical activity).

Group II: organic disease-positive with a low SDS score

The diagnoses in this group were eight cases of Ménière's disease, three cases of vestibular neuritis, two cases of delayed endolymphatic hydrops, one case of unilateral peripheral vestibular dysfunction, one case of bilateral peripheral vestibular dysfunction, one case of

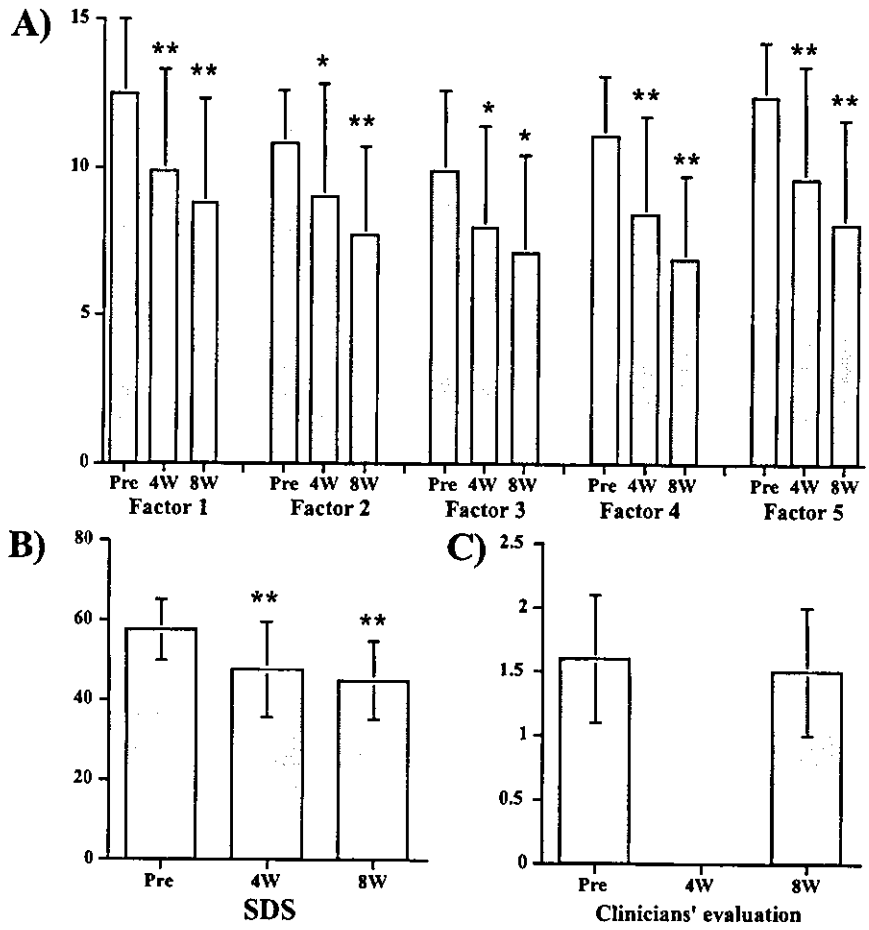

FIG. 1. Subjective handicaps (*A*), SDS scores (*B*), and clinicians' evaluation (*C*) before (pre) and 4 weeks (4W) and 8 weeks (8W) after the SSRI in Group I (organic disease-positive with a high SDS score). Factor 1, disturbance of social activity; Factor 2, body motion precipitating dizziness; Factor 3, limitation of physical activity; Factor 4, emotional disturbance; and Factor 5, disturbance of interpersonal communications. Data are mean ± SD. All five subjective handicaps factors and SDS scores showed improvement by 4 weeks.

benign paroxysmal positional vertigo, and one case of post-head trauma dizziness. As shown in Figure 2B, there was no difference in the SDS score of this group before medication (37.8 ± 6.9) and at 8 weeks after the start of SSRI (38.4 ± 8.7). None of five subjective handicap factors due to dizziness improved after 8 weeks of medication (Fig. 2A). Evaluation by clinicians revealed no improvement (Fig. 2C).

Group III: organic disease-negative with a high SDS score

In this group, no organic diseases were found, but the patients with high SDS scores complained of dizziness and unsteadiness. Five of six patients in Group III showed a decrease in the SDS scores 8 weeks after the start of medication. As shown in Figure 3B, the SDS score 8 weeks after the start of paroxetine (39.0 ± 7.1) was significantly lower compared with that before medication (53.8 ± 5.4) ($p = 0.0431$). Factor 1 (disturbance of social activity) of the self-assessment of handicaps in daily life also showed significant improvement after 8 weeks of medication with paroxetine (Wilcoxon signed ranks test, $p = 0.0431$) (Fig. 3A). Factors 2 to 5 also tended to be improved by the medication, although it did not reach a statistically significant level (Fig. 3A). Evaluation by clinicians revealed no constant abnormal findings (Fig. 3C).

Group IV: organic disease-negative with a low SDS score

In this group, no organic diseases were found, but the patients with low SDS scores complained of dizziness. As shown in Figure 4B, there was no difference in the SDS score 8 weeks after the start of paroxetine (34.3 ± 8.5) and that before paroxetine (37.3 ± 7.5). None of the five subjective handicaps factors showed improvement 8 weeks after the start of paroxetine (Fig. 4A). Evaluation by clinicians revealed no constant abnormal findings (Fig. 4C).

DISCUSSION

SDS score and subjective handicaps in daily life due to dizziness before paroxetine

Of the 47 patients in the current study, 18 patients (Groups I and III) showed a high SDS score (≧48) that exceeded the normal range before medication. Diagnosis of depression should be made by psychiatrists based not only on SDS scores but also on careful interviews, and it is not necessarily meant that patients with high SDS

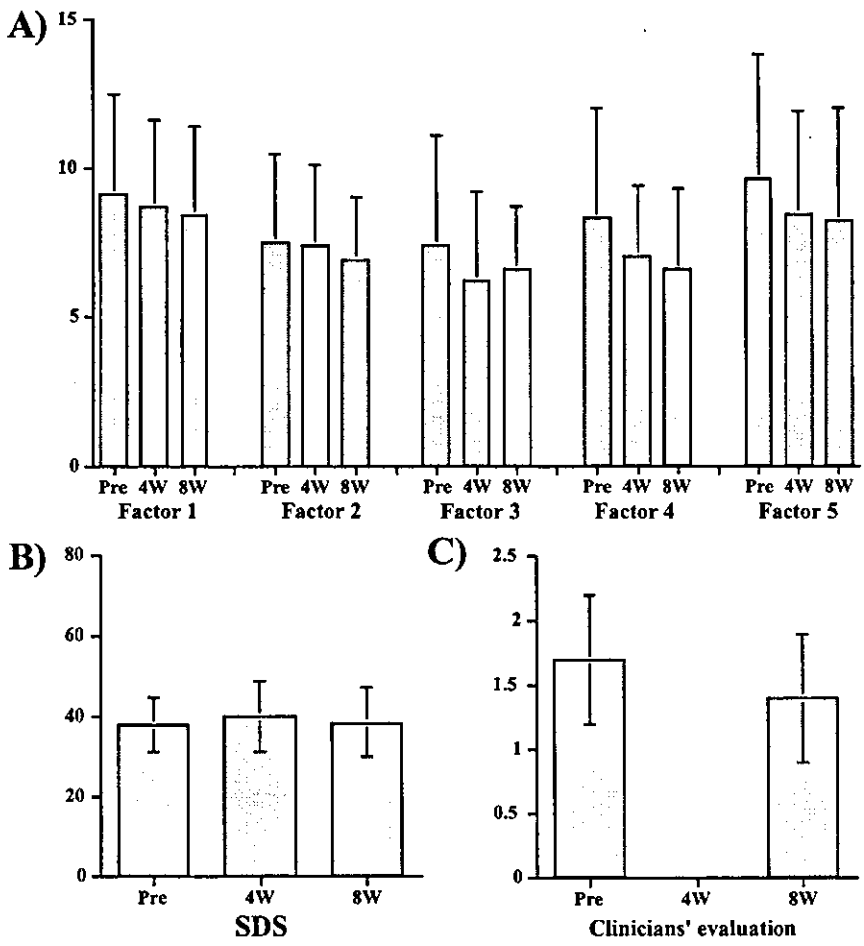

FIG. 2. Subjective handicaps (*A*), SDS scores (*B*), and clinicians' evaluation (*C*) before (pre) and 4 weeks (4W) and 8 weeks (8W) after the SSRI in Group II (organic disease-positive with a low SDS score). Factor 1, disturbance of social activity; Factor 2, body motion precipitating dizziness; Factor 3, limitation of physical activity; Factor 4, emotional disturbance; and Factor 5, disturbance of interpersonal communications. Data are mean ± SD. All subjective handicaps factors, SDS scores, and clinicians' evaluations showed no improvement by 8 weeks.

scores were experiencing depression. However, in accordance with previous reports (1–3), it could be said that patients complaining of dizziness tended to be depressive compared with normal symptom-free adults. This depressive state in dizzy patients may be due to a primary psychiatric disorder or a consequence of vestibular dysfunction.

Subjective handicaps in daily life due to dizziness were assessed using the dizziness and unsteadiness questionnaire. The questionnaire consisted of five factors that could be affected by vertigo and dizziness in daily life: Factor 1, disturbance of social activity; Factor 2, body motion precipitating dizziness; Factor 3, limitation of physical activity; Factor 4, emotional disturbance; and Factor 5, disturbance of interpersonal communications. As shown in Table 3, ANOVA followed by the Bonferroni/Dunn post hoc test showed that all five subjective handicaps factors before medication in Group I (organic disease-positive with a high SDS score) were significantly higher compared with Group IV (organic disease-negative with a low SDS score). It is suggested that those patients primarily having organic diseases who developed a depressive state later had the most difficulties in daily life due to dizziness (Group I). In contrast, those who complained of dizziness without any evidence of organic disease and depression showed fewer difficulties in daily life (Group IV). Therefore, overall, the subjective handicaps assessed by the questionnaire gave us reasonable data on the status of the patients.

Group I: organic disease-positive with a high SDS score

The SDS scores of this group showed a significant decrease after the SSRI, indicating that the SSRI relieved the depressive state in Group I patients, in which various organic diseases as well as a depressive state were apparent. Along with this decline in the SDS score, all five subjective handicaps factors evaluated by the questionnaire significantly improved after 4 weeks of medication, suggesting that the SSRI gave subjective satisfaction in daily life to Group I patients. The decrease in the SDS score showed a significant correlation with improvement of Factor 1 (disturbance of social activity), Factor 4 (emotional disturbance), and Factor 5 (disturbance of interpersonal communications), but not with Factor 2 (body motion precipitating dizziness) and Factor 3 (limitation of physical activity). These results clearly indicate that the SSRI resolved the depressive state in Group I patients and also affected subjective handicaps due to dizziness, especially those related to emotional factors

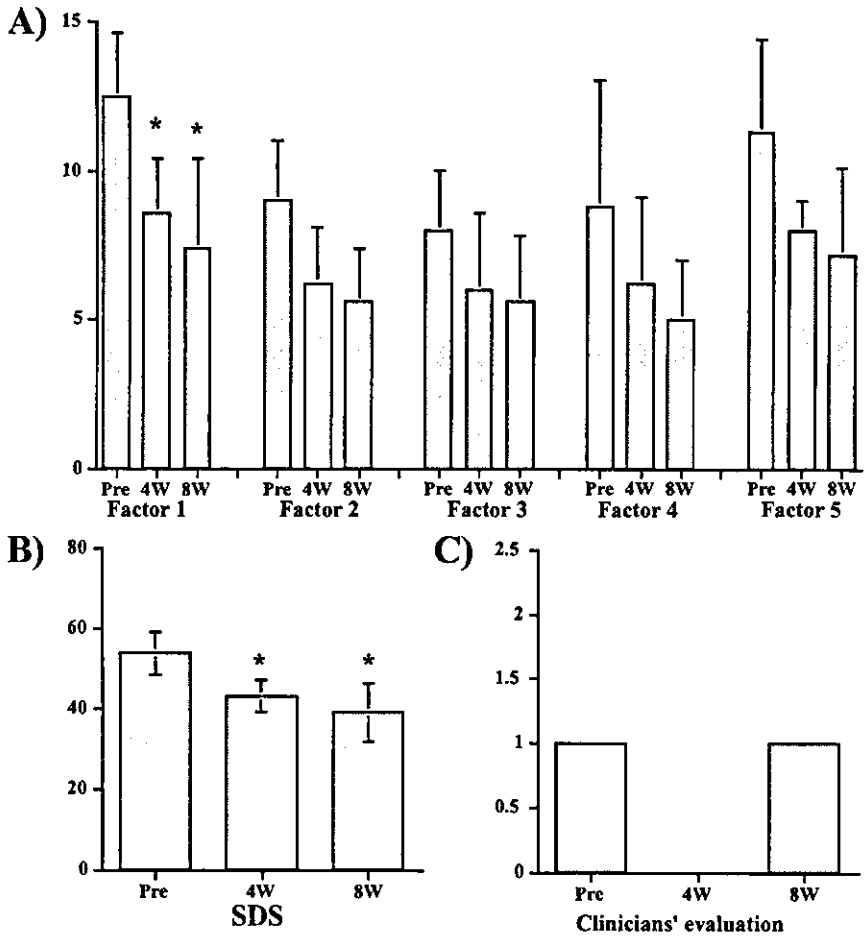

FIG. 3. Subjective handicaps (*A*), SDS scores (*B*), and clinicians' evaluation (*C*) before (pre) and 4 weeks (4W) and 8 weeks (8W) after the SSRI in Group III (organic disease-negative with a high SDS score). Factor 1, disturbance of social activity; Factor 2, body motion precipitating dizziness; Factor 3, limitation of physical activity; Factor 4, emotional disturbance; and Factor 5, disturbance of interpersonal communications. Data are mean ± SD. Factor 1 (disturbance of social activity) and SDS scores showed improvement by 4 weeks.

(Factor 1, 4, and 5). In contrast, the decline in the SDS scores brought about by the SSRI had no correlation with improvement of Factors 2 and 3, probably because the SSRI had no effects on organic diseases and patients still had dysfunction of vestibulo-ocular and vestibulospinal reflexes 8 weeks after the start of medication. The SSRI had effects on both depression and anxiety (4). Therefore, beneficial effects on the anxious state, which sometimes coexists in dizzy patients, would also contribute to the recovery of subjective handicaps. It is also reported that in a mouse model of anxiety, balance control and postural abilities were disturbed compared with a nonanxious strain, and diazepam or an SSRI improved their performance (10,11). Vestibular dysfunction sometimes causes psychiatric disorders, which in turn worsens the functional status of vestibular patients. SSRIs may act to inhibit this "vicious circle" in patients having organic diseases with a high depressive state and probably with other psychiatric disorders including anxiety.

Group II: organic disease-positive with a low SDS score

The mean SDS score of this group before medication was 37.8 ± 6.9, and that at 8 weeks after medication was 38.4 ± 8.7. These results indicate that the SSRI had no effects on the SDS score in nondepressive patients. In this group, none of five subjective handicaps factors due to vertigo and dizziness improved. Objective evaluation by clinicians also did not indicate recovery from the disease. These results suggest that proper medication/rehabilitation for the organic diseases rather than the treatment with an SSRI are required for the improvement of subjective handicaps due to dizziness in this group. Long-term follow-up of psychiatric aspects in patients with long-lasting organic diseases (e.g., vestibular neuritis) in this group and prophylactic effects of SSRIs on induction of the depressive state may be an interesting issue.

Group III: organic disease-negative with a high SDS score

In this group, no organic diseases were found, but the patients with high SDS scores complained of dizziness and unsteadiness. Some of these patients may have had major depression, although diagnosis of depression requires careful interviews by psychiatrists. The SDS score of this group before medication was 53.8 ± 5.4, and that 8 weeks after the start of medication (39.0 ± 7.1) was normal (<48). Although the number of patients in this group was small (n = 6), this decrease in the SDS score was significant, indicating that the SSRI relieved the depressive state in Group III. Factor 1 (disturbance of so-

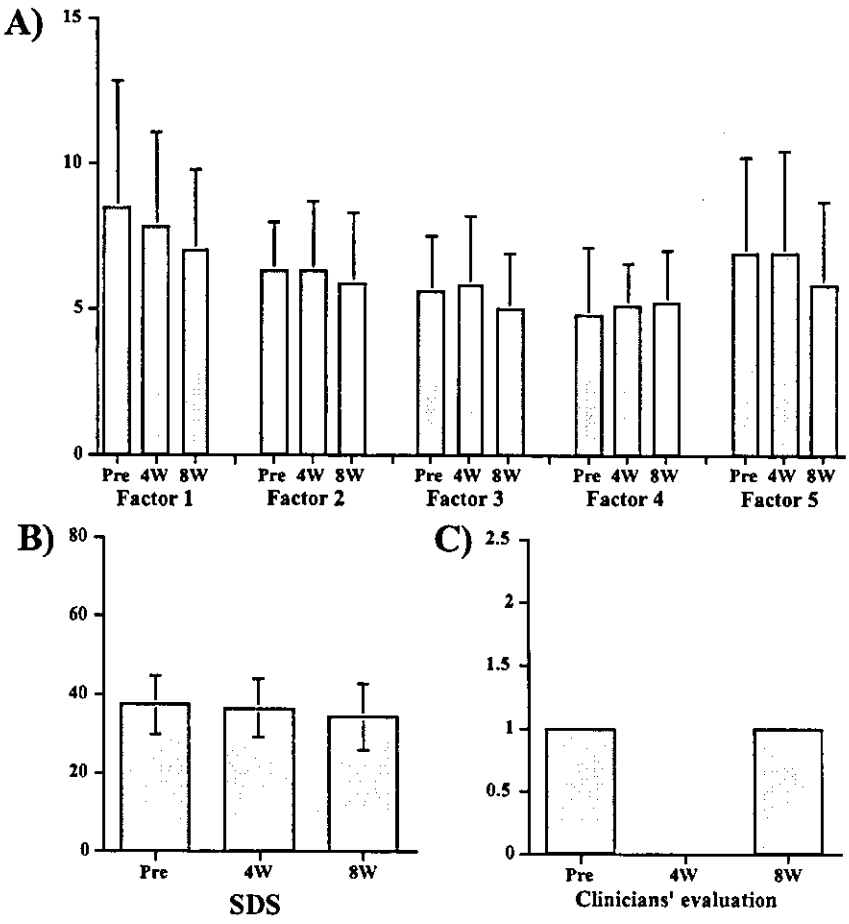

FIG. 4. Subjective handicaps (*A*), SDS scores (*B*), and clinicians' evaluation (*C*) before (pre) and 4 weeks (4W) and 8 weeks (8W) after the SSRI in Group IV (organic disease-negative with low SDS scores). Factor 1, disturbance of social activity; Factor 2, body motion precipitating dizziness; Factor 3, limitation of physical activity; Factor 4, emotional disturbance; and Factor 5, disturbance of interpersonal communications. Data are mean ± SD. All subjective handicaps factors and SDS scores showed no improvement by 8 weeks.

cial activity) of the self-assessment of handicaps in daily life also showed significant improvement after 4 weeks of medication with the SSRI. We initially hypothesized that the SSRI would be more beneficial to this group (organic disease-negative with a high SDS score) than to Group I (organic disease-positive with a high SDS score), because the SSRI would not resolve the vestibular dysfunction in Group I. Although improvement of subjective handicaps in Group III is limited to Factor 1 (disturbance of social activity), the SSRI had an effect on all five factors in Group I. We thought that this was probably due to the small number of patients in Group III and not due to a substantial difference in depressive state between Group I and Group III. Indeed, the pattern of the decline in subjective handicaps brought about by the SSRI in Group III (Fig. 3) was very similar to that of Group I (Fig. 1), and addition of patients in Group III would be expected to cause a more significant decline in subjective handicaps.

Group IV: organic disease-negative with a low SDS score

In this group, no organic diseases were found, but the patients complained of dizziness. The SDS score of this group before medication was 37.3 ± 7.5, and that 8 weeks after the start of medication was 34.3 ± 8.5. Neither SDS score nor all five factors evaluated by the dizziness and unsteadiness questionnaire showed improvement as a result of the SSRI. These results could be interpreted as meaning that unknown organic diseases might have existed in this group and gone unnoticed by clinicians, and that the patients in this group may not have been treated properly. All five scores of subjective handicaps in daily life before medication in this group were significantly lower compared with Group I (organic disease-positive with a high SDS score). There arises another possibility that the assessment of subjective handicaps in daily life using the dizziness and unsteadiness questionnaire is not sensitive enough to detect a small recovery in symptoms in patients with just mild complaints in Group IV. As another possibility, 20 mg/d of paroxetine may have been too low for these patients, because patients with chronic, moderate symptoms often require higher doses of SSRIs than those with more acute illness in the treatment of mood and anxiety disorders.

CONCLUSIONS

Because there seems to be a large number of dizzy patients with a depressive state, the psychiatric status should be addressed in the treatment of dizziness. SSRIs

are useful and effective drugs for obtaining subjective satisfaction in dizzy patients, and specifically in those with high SDS scores. To date, only one report on the beneficial effects of SSRIs on dizziness with psychiatric disorders is available (5). In this article, SSRIs were reported to be effective for dizziness with both major and minor anxiety disorders (5), whereas SSRIs had beneficial effects only for patients with high SDS scores in our patients. This is probably due to the different methods for assessment used and different psychiatric symptoms focused on (anxiety versus depression). Although the current study has several limitations such as unblinded design without a placebo control group, the main conclusion of the current study was the same as that of the previous report (5), and it is reasonable to conclude that SSRIs are favorable for dizzy patients, specifically, those with high SDS scores.

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