

# Benzodiazepine Receptor Deficiency and Tinnitus

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**Abstract:** As regards the symptom of a predominantly central tinnitus of the severe, disabling type, it has been hypothesized that a deficiency in the benzodiazepine receptor exists in the medial temporal lobe system of brain and is directly related to affect impairments including anxiety, stress, depression, and fear. This hypothesis has been investigated with single-photon emission computed tomography using the benzodiazepine radioligand <sup>123</sup>I Iomazenil.

Visual analysis revealed preliminary results of diminished benzodiazepine-binding sites in the medial temporal cortex of all patients with severe tinnitus (N = 6), a finding that is consistent with the hypothesis implicating GABAergic mechanisms in the pathophysiology of the disorder. An abnormal  $\gamma$ -aminobutyric acid-A benzodiazepine receptor density may be an objective neurochemical measure of the severity of a central-type tinnitus and a rationale for treatment. Clinical correlation with the history, clinical course of the patient, and stress questionnaire are presented.

**Key Words:** affect; benzodiazepine receptor; final common pathway; <sup>123</sup>I Iomazenil; medial temporal lobe system; sensory; SPECT imaging of brain; stress

Tinnitus is a disorder of auditory perception due to an altered state of excitation or inhibition in neuronal networks that results in a dysynchrony in neuronal signaling (1992) [1-4]. The pathophysiology of tinnitus is inadequately understood, although alterations in normal GABAergic function have been implicated in patients with the symptom of a predominantly central, subjective, idiopathic tinnitus of the severe, disabling type, in part due to the observation of a lessening of the symptom while the patient is on  $\gamma$ -aminobutyric acid-active (GABA-A) benzodiazepine medications. GABA, as measured by the GABA-A receptors, is the major inhibitory neurotransmitter of brain. Benzodiaz-

epines bind to these GABA-A receptors. Abnormalities of these central benzodiazepine receptors have previously been identified in neuropsychiatric conditions including epilepsy [5-7], Alzheimer's disease [8], Huntington's chorea [9-11], schizophrenia [12-14], and stress [15-17]. In addition, significant deterioration in normal affective functioning is a key feature of severe tinnitus patients and manifest by increasing difficulties with emotional regulation, anxiety, stress, depression, and fear. A specific abnormality of benzodiazepine function is hypothesized to exist in the medial temporal lobe system (MTLS) of brain in severe central tinnitus, as it is this area that influences sensory or affect transformation [18,19].

The tracer <sup>123</sup>I Iomazenil is a benzodiazepine receptor analog single-photon emission computed tomography (SPECT) tracer that has previously demonstrated an accurate measure of intracerebral regional GABA activation [20-23; JP Seibyl, personal communication, June 1999]. We propose to compare in tinnitus patients

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the  $^{123}\text{I}$  Iomazenil SPECT brain distribution and the normal density pattern of the GABA benzodiazepine receptor as an objective neurochemical measure (i.e., a biochemical marker) of the severity of a predominantly central tinnitus and as a rationale for treatment.

$^{123}\text{I}$  Iomazenil binding to benzodiazepine receptors has been measured in human brain using kinetic and equilibrium methods. The studies demonstrated the feasibility of quantifying receptor binding with SPECT [20–23]. Kinetic and equilibrium methods provided adequate measures of regional total equilibrium distribution of  $^{123}\text{I}$  Iomazenil. The SPECT results were considered to compare favorably with positron emission tomography measurements of benzodiazepine receptors in terms of absolute values and identifiability of parameters.

## METHOD

### Patient Selection

Six patients with a predominantly central tinnitus of the severe, disabling type were selected for this study. Each patient reported that his or her subjective idiopathic tinnitus was severe and disabling and had been problematic for longer than 1 year. Three patients were male and three female (Table 1). Four patients were right-handed and two left-handed. Each patient completed a medical-audiological tinnitus patient protocol (MATPP) in an attempt to establish an accurate tinnitus diagnosis [24]. All reported a bilateral tinnitus that was more intense in one ear than the other (see Table 1). Three reported that the symptom was not confined to their ears but also was located in the head. The masking curves were consistent with a predominantly central tinnitus ( $N = 5$ ) (see Table 1). A gadolinium-enhanced magnetic resonance imaging (MRI) scan of brain and SPECT im-

aging of brain with technetium  $^{99\text{m}}$  hexamethyl propyleneamine oxine ( $^{99\text{Tc}}$ -HMPAO) had been performed in the past and revealed perfusion asymmetries. No structural abnormalities were reported on MRI of brain. All subjects had functional asymmetries on SPECT imaging of brain, highlighted by perfusion asymmetries in the MTLs.

Outcome data included a stress questionnaire, depression questionnaire, and tinnitus handicap inventory completed by each patient prior to this study and were consistent with a diagnosis of tinnitus of the severe, disabling type (Table 2). Psychiatric consultation and treatment for anxiety or depression were ongoing for each patient included in this study. Consent forms were discussed with each patient and included an invitation to participate, description of the project, description of procedures, explanations of risks and inconveniences, benefits, economic considerations, confidentiality, coverage in case of injury, and the voluntary nature of participation. Ample opportunity and time to ask questions were provided to patients.

### Study Procedures

#### Screening Visit

Each subject completed a preliminary evaluation within 60 days of study entry. Informed consent was signed before screening procedures were performed [JP Seibly, personal communication, June 1999]. The evaluation included (1) inclusion and exclusion criteria; (2) medical and neurological history; (3) physical examination; (4) supine and standing blood pressure and pulse; (5) 12-lead electrocardiogram; and (6) screening laboratory tests, including a complete blood cell count, chemistries (Na, K, Cl,  $\text{HCO}_3$ , blood urea nitrogen, creatinine, glucose, Ca, phosphorus, lactate dehydrogenase, alkaline

**Table 1.** Demographic, Neurootological, Audiometric, and Neuroradiological Data on Subjects

Patient	Age (yr)	Gender	Hearing Loss?	Location	CTT	Brain MRI	Brain SPECT HMPAO	FMC
HRO 438	58	M	Yes	Ear L > R	1° Central; 2° cochlear	Negative	Abnormal	Type IV IPSI R-L
HRO 439	55	F	Yes	Ears R & L	1° Central; 2° cochlear	Negative	Abnormal	Type I IPSI R; Type IV IPSI L
HRO 440	64	M	Yes	Ear R > L Head	1° Central	Negative	Abnormal	Type IV IPSI R
HRO 441	70	F	Yes	Ear L > R Head	1° Central	Negative	Abnormal	Type IV IPSI R-L
HRO 443	38	M	Yes	Ear L > R Head	1° Central; 2° cochlear	Negative	Abnormal	Type IV IPSI R-L
HRO 445	53	F	Yes	Ear R (L?)	1° Central; 2° cochlear	Negative	Abnormal	Type III IPSI R-L

1° = primary; 2° = secondary; F = female; FMC = Feldmann masking curves; HMPAO = hexamethyl propyleneamine oxine; IPSI = ipsilateral; L = left; M = male; MRI = magnetic resonance imaging; R = right; SPECT = single-photon emission computed tomography.

**Table 2.** Outcome Questionnaires Administered Before and After  $^{123}\text{I}$  Iomazenil SPECT Brain Imaging

Patient	Age (yr)	Date	TST	TII	Depression (Zung)	THI	Date of $^{123}\text{I}$ SPECT
HRO 438	58	1/26/99	31 (mild)	5-7	32/40 (normal)	20 + 20 + 8 = 48 (moderate)	4/27/99
HRO 439	55	2/09/99	34 (moderate)	5-7	38/47.5 (normal)	26 + 12 + 12 = 50 (moderate)	4/27/99
HRO 440	64	3/30/99	37 (moderate)	5	36/45 (normal)	14 + 16 + 10 = 40 (moderate)	5/4/99
HRO 441	70	4/20/99	42 (moderate)	5-7	35/43.75 (normal)	28 + 20 + 14 = 62 (moderate)	5/3/99
HRO 443	38	6/8/99	64 (severe)	6-7	54/67.5 (abnormal, severe)	36 + 32 + 14 = 82 (moderate)	8/10/99
HRO 445	53	9/21/99	44 (moderate)	6-7	42/52.5 (normal)	22 + 10 + 8 = 40 (moderate)	9/28/99

SPECT = single-photon emission computed tomography; THI = tinnitus handicap inventory; TII = tinnitus intensity index; TST = tinnitus stress test.

phosphatase, creatine phosphokinase, bilirubin, total protein, and albumin levels), and a urinalysis.

### Imaging Visit

The six subjects who met the inclusion criteria and who did not meet the exclusion criteria were enrolled into the study after they agreed to participate and signed the informed consent for the designed protocol. Before each injection, subjects were pretreated with stable iodine (10 drops of a saturated solution of potassium iodide) to reduce the uptake of  $^{123}\text{I}$ . Subjects had five external plastic fiducial markers containing 1 to 4  $\mu\text{Ci}$  of  $^{99\text{m}}\text{Tc}$  affixed along the canthomeatal line for post hoc realignment of the SPECT images. Next, subjects were injected with 8 to 10 cm of  $^{123}\text{I}$  Iomazenil administered as a bolus plus constant infusion over approximately 6 hours using previously published methods. The purpose of the constant infusion was to obtain an equilibrium-binding condition at the brain benzodiazepine receptor. Under such conditions, analysis of the SPECT signal provided an outcome measure linearly related to the density of benzodiazepine receptors, unaffected by brain blood flow effects on the delivery of tracer to brain binding sites. SPECT images were acquired on the PICKER PRISM 3000XP camera (Marconi Medical, Cleveland, OH).

The following safety measures were applied for each patient: Vital signs (temperature, pulse, respirations, and blood pressure) were measured and recorded immediately prior to and at 15 minutes and 30 minutes after the  $^{123}\text{I}$  Iomazenil injection and again prior to obtaining the scan. Adverse events were monitored and logged in the U.S. Food and Drug Administration-approved case report forms.

### Data Analysis

#### Data Acquisition

Projection data were acquired on the PICKER PRISM 3000 camera fitted with high-resolution fan-beam collimators (acquisitions obtained for 60 minutes,  $128 \times 128$  matrix) into a 20% symmetrical window centered

on 159 keV [JP Seibyl, personal communication, June 1999]. Raw projection data were obtained with a Butterworth filter (power = 10; cut-off = 0.26 cm), and a filtered backprojection was performed with a simple ramp filter. These processing filters were optimal for quantitative assessment. All subjects had an MRI scan for coregistration with the SPECT images and accurate region-of-interest placement.

#### Image Analysis

Individual SPECT scans were registered to the patient's MRI scan using previously published methods [25]. Visual analysis of the images was performed by two nuclear medicine physicians blinded to clinical information about patients. Regions of interest were drawn using an identical circular region of interest ( $1,467 \text{ mm}^3$ ) in standardized locations in the hippocampus, frontal lobes, thalamus, cerebellum, and frontal region. The frontal lobe regions were designated the a priori controls. Using the count density data derived from these regions, a left-right asymmetry ratio was developed for each region. In addition, the absolute percentage difference for either the right or left homotypic regions was determined. Both left-right homotypic ratios and absolute percentage difference statistical comparisons between brain regions and frontal control were assessed using non-parametric statistics (Mann-Whitney U test). Statistical significance was assessed at the  $p < .05$  level (two-tailed).

#### Imaging: Quality Control

Quality control of the SPECT imaging system included uniformity correction flood and center-of-rotation (COR) determination, performed prior to imaging of subjects. A one-time 120-million-count ( $128 \times 128$  matrix) extrinsic uniformity correction flood was acquired using  $^{123}\text{I}$  for SPECT reconstruction in a fillable phantom.

## RESULTS

### Visual Analysis

Visual analysis of the  $^{123}\text{I}$  Iomazenil SPECT images by two board-certified nuclear medicine physicians sug-

gested consistent areas of diminished tracer uptake in five of six patients (Fig. 1), three in the medial left temporal lobe and two in the medial right temporal lobe. One patient had more subtle but probable changes in the medial left temporal lobe. On the basis of visual analysis, other brain regions, including other cortical regions and cerebellum, did not demonstrate clear relative changes in Iomazenil uptake. Subcortical structures, including basal ganglia and thalamic nuclei, showed low uptake consistent with the known lower density of benzodiazepine-binding sites in these regions.

### Homotypic Brain Ratios

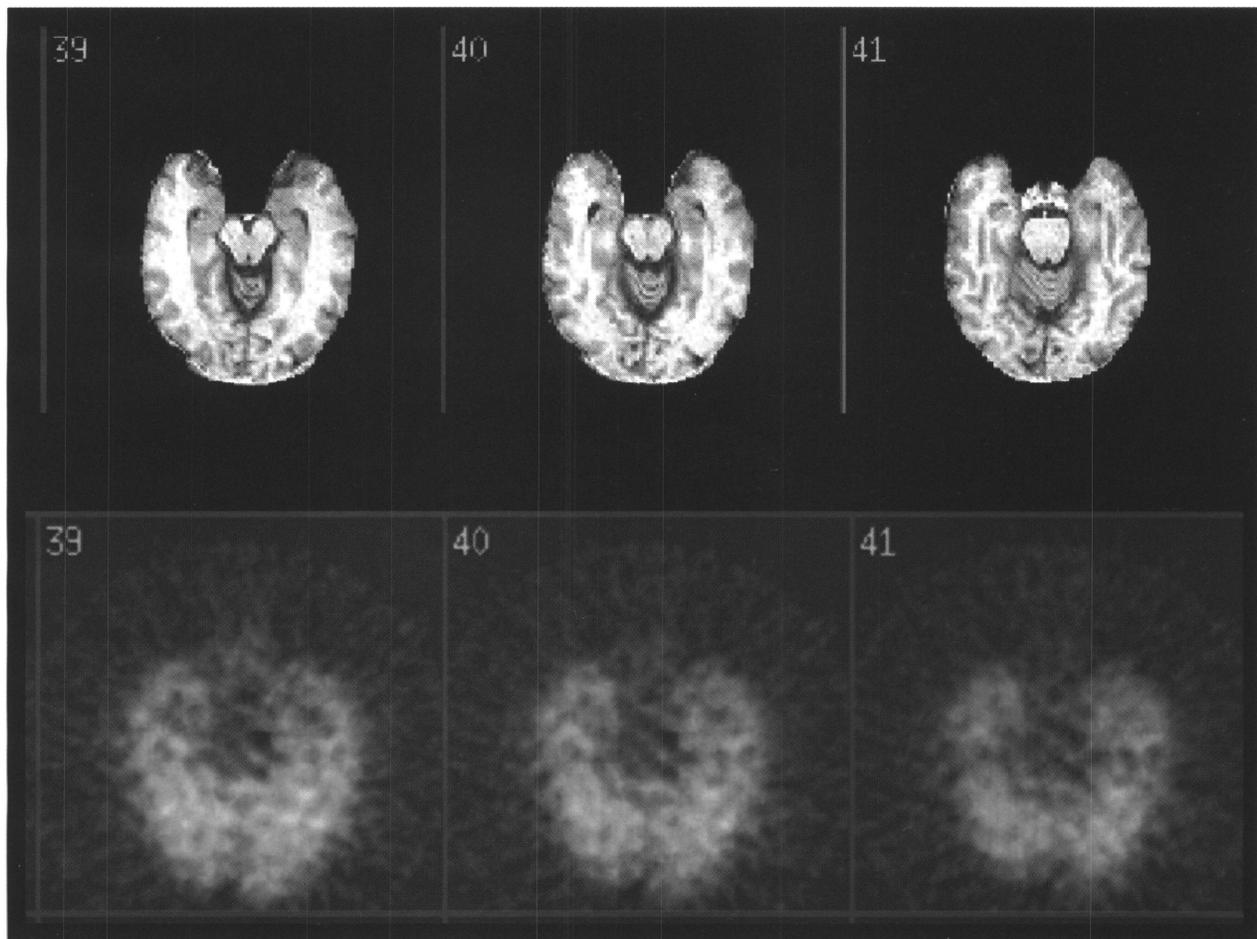
Regions of interest, drawn on standardized brain regions identified on MRI and transferred to coregistered SPECT images in patients, were used to develop left-right homotypic brain ratios (Fig. 2). Assuming the left-right asymmetry as the a priori control region, ratios for superior temporal gyri were significantly more asym-

metrical relative to frontal lobes ( $p = .0411$ , two-tailed). Comparisons of the left-right ratio for hippocampus and cerebellum were not statistically different from frontal control ratios ( $p = .394$  and  $p = .589$  for hippocampus and cerebellum, respectively).

Figure 3 indicates another measure of regional brain asymmetry based on the absolute percentage differences for the same regions as were analyzed previously. The Mann-Whitney U test demonstrates a nonsignificant trend ( $p = .093$ , two-tailed) for hippocampus as compared with frontal cortex but no significant differences on this measure for superior temporal lobe ( $p = .310$ ) or cerebellum ( $p = .204$ ) relative to frontal cortex.

### SPM Analysis

A multistep statistical process was used to realign and coregister all the cases and controls to one another and to incorporate them into a standard spatial volume (Talairach space). Control subjects ( $n = 12$ ) were age- and



**Figure 1.** Coregistered magnetic resonance images (top panel) and  $^{123}\text{I}$  Iomazenil single-photon emission tomographic images in chronic central tinnitus patient demonstrates diminished medial left temporal lobe density of benzodiazepine binding sites.

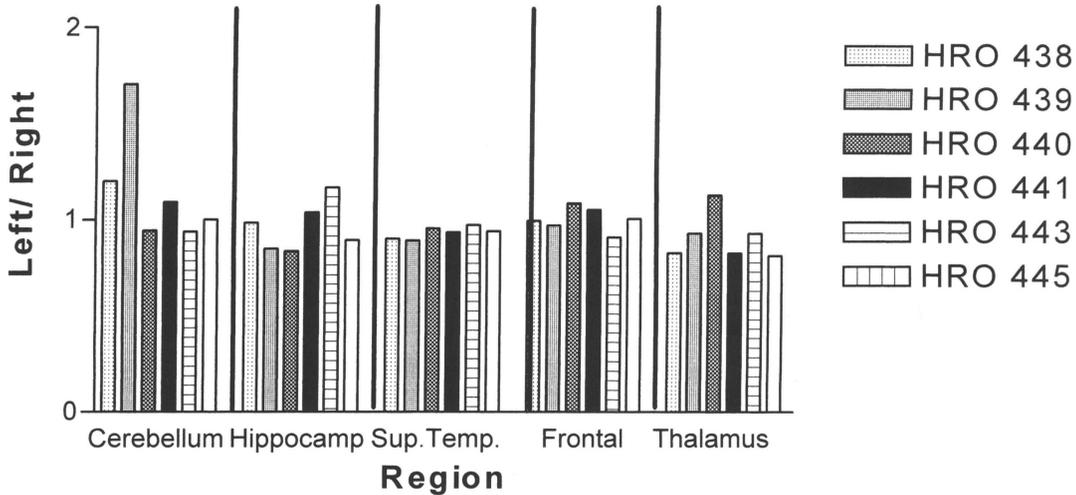


Figure 2. Left-right homotypic brain ratios in six patients with severe, disabling central tinnitus undergoing <sup>123</sup>I Iomazenil single-photon emission tomographic image scanning. Nonparametric statistical assessment demonstrates significantly greater left-right asymmetry in the superior temporal lobe relative to the frontal control region ( $p = .0411$ , two-tailed).

gender-matched healthy subjects with no history of neurological or psychiatric illness based on physical examination and a history obtained by a research psychiatrist. Using a standard statistical software package—SPM—the mean and standard deviations of each pixel coordinate of the case and control groups were calculated and, subsequently, a paired Student’s *t*-test was performed in which each pixel coordinate of the cases was compared to each pixel coordinate of the controls. Areas with significantly increased or decreased activity in controls as compared to case subjects were displayed on an SPM brain map.

In the SPM case-control analysis, a statistically significant decrease in benzodiazepine receptor density in the cerebellar region was noted, particularly on the right ( $p = .054$ ). The results of this analysis provided support for the results reported on visual analysis.

**DISCUSSION**

Brain SPECT perfusion imaging demonstrated for the first time in 1991 the in vivo significance of the organicity of brain for a predominantly central tinnitus

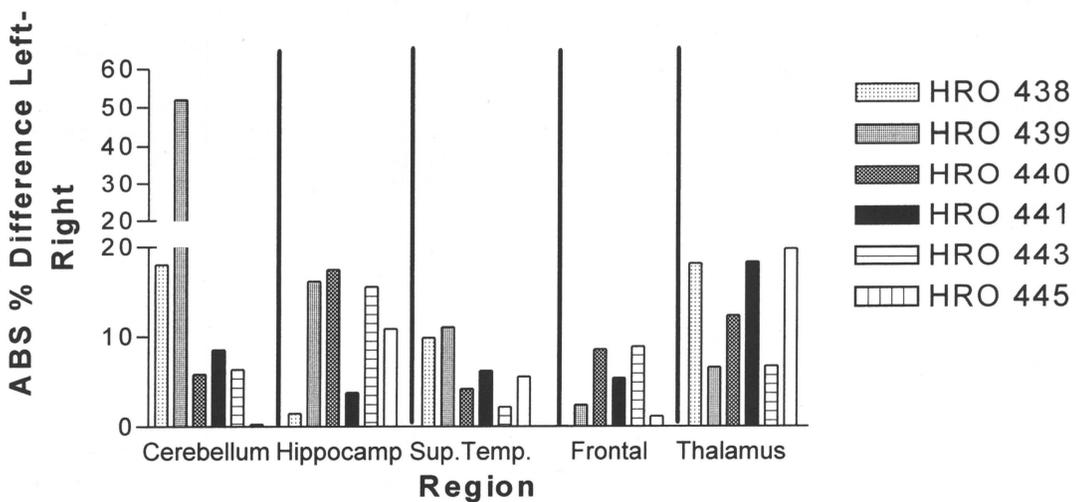


Figure 3. Absolute percentage (ABS %) differences for selected homotypic brain regions based on the magnetic resonance imaging-derived regions of interest used in Figure 2.

[1,4]. Neurootological and neurological implications, including cerebrovascular disease, neurodegenerative disorders, dementia, and neuropsychiatric mood disorders, were suggested [26]. SPECT of brain with the radioisotope  $^{99}\text{Tc}$ -HMPAO identified persistent side-to-side perfusion asymmetries highlighted by the amygdala hippocampal complex of the MTLs [1,4,27,28,29,30]. Adjacent perfusion asymmetries involving the frontal, temporal, and parietal lobes suggested an inter-neuronal network that could result in the transition of the sensory to the affect components of the symptom of tinnitus. These findings are common in all patients who are submitted to SPECT imaging of brain. These results led the authors to believe that a final common pathway exists for all patients with tinnitus, particularly tinnitus of the severe, disabling type, which we have studied. Within the final common pathway, the amygdala-hippocampus structures of the MTLs are particularly critical in the development of what we believe is a paradoxical auditory memory [28–30].

Tinnitus is hypothesized also to be a consequence of an alteration in auditory masking resulting from regional diminution of neural inhibition. If this is true, the event probably is mediated by reduced regional GABA release. Theoretically, blockage of GABA-mediated inhibition results in tinnitogenesis, an epileptiform auditory phenomenon [4,26].

## BACKGROUND

Since 1977, more than 6,000 patients with subjective, idiopathic, severe, disabling tinnitus have completed the MATPP in the Tinnitus Clinic of the Health Sciences Center at Brooklyn, State University of New York [3,24]. From 1989 through 1999, SPECT imaging of brain has been performed on 95 selected patients identified on the MATPP to have a predominantly central-type tinnitus. In 49 patients, an acetazolamide (Diamox) stress test was completed. The patients selected for this study demonstrated no evidence of central nervous system disease either by clinical history or physical examination. Results of gadolinium-enhanced MRI studies of brain in all patients were reportedly negative.

We have observed that benzodiazepines (e.g., clonazepam [Klonopin], alprazolam) have been reported to influence the symptom of tinnitus significantly [31,32]. Our results for tinnitus relief were reported with Klonopin since 1984 [31,33]. In general, Klonopin-treated tinnitus patients emphasized that primarily the affect component of the tinnitus symptom was influenced: That is, they reported improved sleep, an increased ability to “cope” with tinnitus, and occasional

reduction in tinnitus intensity. The Alprazolam-treated tinnitus patients reported reduction in tinnitus, improvement in affect, and occasional hearing improvement [32].

The adverse influence of stress has been found, in our experience, to be practically universal for all tinnitus patients [34]. The stress diathesis model for depression explains the relationship between increasing levels of change in affect, highlighted by anxiety and depression, and increasing levels of cortisol. The resultant hypercortisolemia acts on the hypothalamic-pituitary axis, though recent reports suggest that the site of the hypercortisolemia lesion, with a consequent increase in anxiety and depression, is not the hypothalamus but the hippocampus, which is part of the MTLs [15–17,35–37].

The stress model for tinnitus is an adaptation of the stress model for depression [38,39]. Theoretically, stress is considered to be a modulator for the final common pathway for tinnitus in the MTLs. It is proposed that for the tinnitus patient, a cycle is established wherein reciprocal projections among hippocampus, amygdala, entorhinal cortex, and hypothalamus vary in the degree of control of cortisol levels that arise during stress and have a resultant influence on the sensory and affect components of tinnitus. Variations in control of cortisol levels result in further strengthening of an already established paradoxical memory, with additional negative affect behavioral manifestations of mood, fear, anxiety, depression, and the like. In time, tinnitus of the severe, disabling type gradually develops.

The concept of the final common pathway for all clinical types of tinnitus embodies the hypothesis that the benzodiazepine receptor and its relationship to GABA-A are considered significant in the transition of the sensory to the affect component of tinnitus. The benzodiazepine receptor's role, particularly with respect to GABA-A and modulation of the GABA-A benzodiazepine receptor by cortisol (especially under stress), is considered to be significant in the clinical course of especially those patients affected by severe, disabling tinnitus [18]. Current data support this role.

The selection of  $^{123}\text{I}$  Iomazenil for SPECT measurement in our study of benzodiazepine receptors was based on experience and a review of the literature [20–23; JP Seibyl, personal communication, June 1999]. SPECT imaging of brain with  $^{123}\text{I}$  Iomazenil has shown significant uptake of the radioligand in a distribution consistent with benzodiazepine receptor binding. In the study, which measured the whole-body distribution of activity after intravenous administration of  $^{123}\text{I}$  Iomazenil, evaluation of organ irradiation confirmed that  $^{123}\text{I}$  Iomazenil has favorable *in vivo* imaging characteristics and that this agent may be useful in assessing disease states in which the function of benzodiazepine GABA-A receptors is abnormal.

The brain uptake of this radiotracer represents reversible and selective binding to the benzodiazepine receptor. The high affinity of Iomazenil for the benzodiazepine receptor is considered to explain in part its high brain uptake and slow washout. Our study allowed the measurement of free parent compound in plasma and examination of the kinetics and clearance of  $^{123}\text{I}$  Iomazenil in human and nonhuman primates.  $^{123}\text{I}$  Iomazenil is cleared from human and nonhuman primate arterial plasma in a triexponential manner. The radioactivity detected in the various regions of brain was almost exclusively  $^{123}\text{I}$  Iomazenil.

The neurochemistry of the GABA receptor is complex [40]. The preliminary findings of our report are considered, on both a basic scientific and a clinical level, to be a forerunner for clinical application of current knowledge about the GABA-glutamate neurotransmitter systems in attempting control of the symptom of predominantly central tinnitus. Our study provides a preliminary, partial understanding of the complex mechanisms underlying the production of different types and subtypes of predominantly central tinnitus. It also attempts to develop a neuropharmacology for control of different clinical types and subtypes of tinnitus. Because the method used is continuous infusion, the effects of regional heterogeneity of blood flow are eliminated.

The results of our study are consistent with the hypothesis implicating GABAergic mechanisms in the pathophysiology of some clinical types of tinnitus. These results support the rationale of treating regional perfusion asymmetries, which reflects a neurochemical basis for tinnitus control directed at restoring homeostasis between the sensory and affect components of tinnitus, which are regulated by the GABA-A benzodiazepine receptor [41]. Specifically, a benzodiazepine (e.g., Klonopin) in combination with a GABAergic drug (e.g., gabapentin [Neurontin]) is employed. Neurontin, an anticonvulsant, is structurally related to the neurotransmitter GABA but does not interact with GABA receptors if it is not converted metabolically into GABA or a GABA agonist, and it is not an inhibitor of GABA uptake or degradation [42].

Significant neurochemical implications for treatment are suggested by the proposed final common pathway for tinnitus [30]. The concept of a final common pathway for tinnitus, which has evolved since 1983 from our clinical experiences with tinnitus, stems from observations that all patients with tinnitus, particularly those with the severe, disabling type, exhibit as a common denominator a disorder in affect [18]—that is, a behavioral disorder in response to and as an accompaniment of an aberrant auditory sensory stimulus. Clinical neuroscientific reports have identified the MTLs' central role in memory, emotion, and behavior [14,43].

Such reports likely are related to the SPECT findings of significant perfusion asymmetries in our tinnitus patients.

The concept of a final common pathway for tinnitus reflects new images of tinnitus—specifically, a neurochemistry of tinnitus [41]. SPECT or positron emission tomography of brain (or both) provide an opportunity to establish a relationship between the clinical manifestations of tinnitus and specific chemical and receptor abnormalities in the anatomic regions of interest. The perfusion asymmetries reflect the clinical course of tinnitus. In situ radioactive tracers are hypothesized to serve as biochemical markers in tinnitus studies and to have the potential for providing direction for drug development and treatment application to alter the tinnitus symptom or disease process. This hypothesis formed the basis for this study, which attempts to establish the role of GABAergic mechanisms in the pathophysiology of tinnitus and to explain the significant efficacy of benzodiazepine therapy, which has been reported clinically to provide tinnitus relief. Our goal was to provide clinical justification for benzodiazepine therapy in tinnitus patients and to attempt to understand the underlying mechanisms involved in the reported improvement in affect or tinnitus intensity. The identification, by use of  $^{123}\text{I}$  Iomazenil SPECT imaging, of diminished benzodiazepine-binding sites in the medial temporal cortex of patients with severe tinnitus is consistent with the final common pathway hypothesis implicating GABAergic mechanisms in the pathophysiology of tinnitus in selected patients and with positive results of benzodiazepine treatment based on speculations about the underlying mechanisms of tinnitus production (i.e., secondary to deficits in the GABA-glutamate neurotransmitter systems [28,30,41]).

### **Benzodiazepine Deficiency Syndrome, Stress, and Tinnitus**

It has been hypothesized that a benzodiazepine deficiency syndrome exists in the MTLs that influences sensory or affect transformation [18,30,44]. The positive effect of benzodiazepine therapy for tinnitus control is hypothesized to be the reestablishment of a benzodiazepine level sufficient to allow normal processing and development of affect, expressed clinically as a restoration of a normal mood with elimination or control of fear, anxiety, or depression. Specifically, when a benzodiazepine is administered to tinnitus patients, the reported tinnitus relief experienced is thought to reflect neurochemical replacement of the deficient benzodiazepine receptor.

Preliminary results of  $^{123}\text{I}$  Iomazenil SPECT imaging have shown diminished benzodiazepine-binding

sites in the medial temporal cortex in patients with severe, disabling tinnitus [JP Seibyl, personal communication, June 1999]. This is consistent with the original hypothesis of a final common pathway for tinnitus, which implicated GABAergic mechanisms in the pathophysiology of some clinical types of tinnitus.

The high concentration of steroid and benzodiazepine receptors in the MTLs of brain was reported by McEwen et al. (15–17,46). It has been reported that the benzodiazepine receptor exerts its effect by attaching to the GABA-A receptor. Steroid receptors are known to modulate GABA-A activity, whereas stress is known to be intermittently involved in cortisol steroid benzodiazepine activity (36,37,47–49). Tinnitus is a stressful experience. Increasing evidence supports an increased occurrence and severity of disease processes with stress, the focus being on alterations in the hippocampus [15–17,45–48].

Glucocorticoids are important in signaling and coordinating a variety of physiological responses, ranging from immune inflammatory processes to promoting the production of energy in the form of carbohydrates and fat [45]. The brain is the principle target for circulating glucocorticoids [46]. One of the most important brain areas responding to circulating adrenal steroids is the hippocampal formation, which contains high concentrations of adrenal steroid receptors [45,47]. Excitation in the hippocampus is regulated by the release of GABA from local neurons [15].

Glucocorticoid secretion is a result of contact with a physical stressor. During aging, sustained stress, and in some neuropsychiatric disorders, glucocorticoid secretion is interrupted. A consequence of glucocorticoid excess is damage to certain neurons in the brain, particularly those in the area of the hippocampus; this has been demonstrated by aging the rat [35]. The defect is in the direction of hypersecretion of glucocorticoids. The consequences of glucocorticoid excess are two-fold: the effect of hippocampus on glucocorticoid secretion and the effect of glucocorticoids on the hippocampus. Recent work has shown that *N*-methyl-D-aspartate (NMDA) receptor blockade is also affected in preventing stress-induced dendritic atrophy [48]. Cortisol or stress may alter CA3 neuronal atrophy by regulating GABAergic synaptic inhibition. It appears that cortisol may alter the excitability of hippocampal neurons via regulation of GABA-A receptor expression. In the future, the question of whether corticosteroid effects on neuronal morphology involve changes in the number of pharmacological properties of the GABA-A receptors needs to be examined [M Orchinik, NG Weiland, BS McEwen, unpublished observations, 1995].

Our study, which focuses on the MTLs of brain, suggests that in the tinnitus patient, the medical signifi-

cance for the patient may be that of “an aging hippocampus” and that tinnitus may be a “soft” sign of aging. The site of lesion in these tinnitus patients may be the hippocampus. This finding supports the hypothesis that GABAergic neurochemical mechanisms play a role in the classic course of tinnitus, particularly of the severe, disabling type.

## Questions

Interesting questions are raised by the results of our study:

1. *Are the findings of diminished benzodiazepine-binding sites reflective of or influenced by prior use of anxiolytic or antidepressant medications?* SPECT brain imaging studies of healthy subjects conducted with Iomazenil demonstrate very minimal effects of alprazolam on brain measurements [49]. In contrast, previous SPECT brain imaging studies conducted with <sup>123</sup>I Iomazenil in a limited number of patients affected with panic disorder and depression suggest decreases in the frontal and parietal lobes [50]. In our study, the predominant benzodiazepine effect was found in the MTLs and cerebellum.

Basic scientific reports describe variations in chronic benzodiazepine effects on both binding sites and function and varying regional specificity. The differential effects of the various drugs may be due to the binding characteristics of the drug in question and to site specificity. Finally, a particular benzodiazepine may affect another neurotransmitter system with indirect effects on the GABA functions of the GABA-A complex. A combination of drugs for tinnitus relief may differentially affect the GABA-A receptor subunit's gene expression.

Chronic benzodiazepine administration has been associated with tolerance and downregulation of GABA-A receptor binding and function. The effects of chronic benzodiazepine administration on the GABA-A receptor may be both region-specific and receptor subtype-specific. Effects of individual benzodiazepines on brain regions have varied. Receptor alterations induced by lorazepam occurred in cortex, hypothalamus, and hippocampus, whereas alterations associated with alprazolam occurred in cortex and hypothalamus only. A possible mechanism for this discrepancy is the differential effects of the two drugs on benzodiazepine receptor subtypes [51]. After chronic diazepam use, regional specificity for GABA-dependent chloride uptake has been identified in the cortex but not the cerebellum [52].

2. *Are the findings of diminished benzodiazepine-binding sites specific for the symptom of tinnitus or reflective of the behavioral (i.e., affective) component of the symptom of tinnitus?* All patients with idiopathic, severe, disabling tinnitus not only are adversely influ-

enced by stress but have a significant alteration in affect manifested by changes in emotion, fear, anxiety, and depression [53]. All patients in our study were adversely influenced by stress and also were being treated by psychiatrists for anxiety or depression. Future investigation is planned to identify whether the findings in this study are specific for the underlying tinnitus sensory component and the behavioral affect response to tinnitus or for the affect response accompanying stress or for stress in general.

3. *Is the finding of diminished benzodiazepine-binding sites clinically specific for the sensory component of tinnitus, with an associated interference in affect?* In the original hypothesis of the final common pathway for tinnitus, a homeostasis in the normal patient between the sensory component and affect was considered to be modulated by stress. It is possible that our findings of diminished benzodiazepine-binding sites not only may reflect the affect component but may explain what has been speculated in the past—namely, that the progressively increasing cortisolemia accompanying stress can result in the symptom of tinnitus (i.e., a sensory complaint). Not infrequently, a significant proportion of tinnitus patients cite a stressful situation (e.g., death of a friend or family member) as being related to the onset of tinnitus. Whether exogenous steroid in a tinnitus patient worsens the condition has not yet been determined.

A recent basic scientific investigation demonstrated that a potential circuitry underlying the normal auditory map may exist in the absence of stimulation but may be functionally inactivated by GABA-A receptor-mediated inhibition [54]. This finding warrants consideration with respect to the relief of the sensory component of tinnitus that is reported after benzodiazepine therapy. The study indicated that changes in patterns of inhibition as well as adjustments in patterns of excitation can contribute to adaptive plasticity in the central nervous system, as has been demonstrated in the external nucleus of the inferior colliculus. The plasticity of auditory spatial representation in the owl brain has been shown to depend not only on new excitatory connections but on the persistence of old connections mediated by inhibition. By combining and overlaying different plasticity mechanisms, one of which is the GABA-A receptor in the auditory pathway, particularly at the external nucleus of the inferior colliculus, the owl was found to be able to adjust its various sensory maps so that they remain in harmony: For example, homeostasis is regulated by the GABA-A receptor mechanism. Whether these findings can be extrapolated to humans is not yet certain.

4. *What is the neurochemical significance of the reduction of benzodiazepine-binding sites as demonstrated by the use of Iomazenil?* Possibly, the reduction

shown on brain SPECT using  $^{123}\text{I}$  Iomazenil does, in fact, reflect an actual reduction in the total number of binding sites. However, it also is possible that the change reflects the binding affinity of the benzodiazepine receptor for Iomazenil. Alternatively, the results reported in this study might reflect an overall reduction in sensitivity to glutamate.

5. *What is the role of neurosteroids on the benzodiazepine receptor, and specifically, on the binding affinity of Iomazenil reported in tinnitus patients in this study?* Discussions of steroid activity as a modulator for the benzodiazepine–GABA-A–chloride receptor have centered primarily around cortisol, its production and regulation. However, the role of neurosteroids also must be considered.

*Neurosteroids* is a term referring to steroids of central origin that are independent of peripheral sources. Seyle [55] reported in 1942 that steroids exhibited anesthetic and anticonvulsant effects. McEwen et al. [46,56] provided evidence that steroids can influence neuronal excitability and that some endogenous neurosteroids may play a role in the regulation of central nervous system excitability. Chronic neurosteroid treatment produces functional heterologous uncoupling at the GABA-A–benzodiazepine receptor complex in mammalian cortical neurons [57]. Significant are metabolites of progesterone and dioxycorticosteroid, which are found in both cerebral cortex and hypothalamus. The elevated levels of these two neurosteroids have been reported to be of sufficient strength to modulate GABA receptors [15,58–60].

6. *What is the effect on the clinical course of tinnitus of prolonged occupancy of GABA-A receptors by such ligands as GABA and benzodiazepine agonists (a condition known as use-dependent regulation of GABA-A receptors)? That is, what are the issues of drug tolerance?* The GABA-A receptor is the major transducer of rapid inhibitory transmission in the vertebrate central nervous system. GABA-A contains binding sites for a number of anxiolytic and hypnotic drugs including benzodiazepine, barbiturates, and neurosteroids. Such drugs act as modulators of the GABA-A chloride ion channel. Prolonged occupancy of GABA-A receptors by GABA or other ligands produces a series of control mechanisms collectively termed *use-dependent regulation*. GABA-A receptors are heterooligomeric proteins that belong to a superfamily of ligand-gated ion channels, which include the nicotinic acetylcholine receptor, glycine receptor, and 5-hydroxytryptamine receptor. Molecular cloning has revealed diverse subunits for GABA-A receptors, which are grouped into four major classes: alpha, beta, gamma, and lambda. The exact composition and subunit stoichiometry of GABA-A receptors in vivo has not yet been established.

GABA-A receptor phosphorylation represents a major form of modification that produces profound changes in receptor function. Such regulation includes mechanisms that have been subdivided into two distinct pathways: GABA-A receptor downregulation and GABA-A receptor upregulation. Culture of cortical neurons treated with GABA-A or benzodiazepines opens the pathway for GABA-A receptor downregulation. This includes desensitization, tachyphylaxis, sequestering endocytosis of subunit polypeptides, the uncoupling of allosteric interactions between GABA and benzodiazepine-binding sites with subunit polypeptide degradation, and repression of subunit gene expression. The end point of GABA-A receptor downregulation, a reduction in receptor number, is postulated to be established initially by degradation of the receptor protein and then to be maintained by a diminished level of de novo synthesis. Benzodiazepine treatment of many animal experimental preparations may elicit only desensitization, sequestration, or uncoupling, without a decline in receptor number. Components of the GABA-A receptor downregulation pathway can also be evoked by chronic administration of GABA-mimetics, benzodiazepines, barbiturates, and neurosteroids in animals. Such downregulation correlates with the establishment of tolerance to and physical dependence on pharmacological effects of these drugs, suggesting a cellular model for this behavior.

Upregulation of GABA-A receptors is observed as one of the neurotrophic actions of GABA, primarily in cultured cerebellar granule cells. Both upregulation and downregulation of GABA-A receptors appear to represent use-dependent pathways for guiding synaptic plasticity in the vertebral central nervous system [61]. Conceivable artifacts can result in the phenomenon of downregulation as a consequence of either GABA treatment (e.g., selection or induction of a new neuronal population) or of underlying receptor desensitization because of incomplete washout of agonist. Chloride flux can also influence the activity of GABA-A benzodiazepine receptor. The downregulation of ligand binding and chloride flux can be prevented by GABA-A antagonists. Both the synthesis and degradation of GABA-A receptors appear to play roles in GABA-A-induced downregulation. Use-dependent receptor sequestration, degradation, and alterations in gene expression may be physiologically important in the tinnitus patient, separate from total uptake or total number of GABA-A receptors identified in this study.

The issue of drug therapy for tinnitus control is further complicated by what is and is not known of GABA-A receptor activity in vivo. Impaired GABA reuptake has been demonstrated in human temporal lobe epilepsy [62]. Prolonged treatment (>24 hr) of cortical

cultures with GABA induces an uncoupling of ligand binding sites on the GABA-A receptor as well as a reduction in site density. Prolonged chronic treatment of cortical neurons reduces the extent of the coupling effect (i.e., an uncoupling occurs to more than one-half the normal amount) [63,64]. The molecular basis for the uncoupling is not known. What is significant is that an uncoupling of GABA-A receptors is induced by prolonged treatment of cortical cultures.

GABA-A receptor agonists or inhibitors of GABA degradation that are chronically administered produce a tolerance to the anticonvulsant effects. The underlying mechanism is not known, but a reduction in GABA synthesis might contribute to this behavioral tolerance [65]. Animals exposed to GABA-mimetic drugs exhibit an inconsistent pattern of effects on the GABA-A receptor. Such inconsistencies may be due to the high levels of endogenous GABA in the brain and low permeability of the blood-brain barrier to GABA and specific GABA-A agonists. Data indicate that normal GABA-A receptor functions decline during prolonged exposures to GABA in vivo. Significantly, the cerebellum has been identified as an area of unique sensitivity to GABA-evoked downregulation in vivo [65].

Benzodiazepine agonists in vitro significantly increase GABA-A receptor currents by increasing the sensitivity of the receptor to GABA. The mechanism of GABA-A receptor uncoupling by benzodiazepines in vitro may be a permanent conformational change in existing receptors, production of an endogenous regulatory GABA-A receptor ligand, a change in phosphorylation, or a displacement of GABA-A receptor subunits. The favored mechanism is a lasting conformational change in existing receptors. In vitro studies must be directed to identifying the role of endogenous GABA in benzodiazepine-evoked downregulation of GABA activity.

GABA-benzodiazepine coupling was evaluated in eight regions of rat brain by the ability of GABA to stimulate 0.5 nM [<sup>3</sup>H]flunitrazepam binding [66]. GABA increased benzodiazepine binding maximally 40% in cerebellum and medulla and at least 25% in the olfactory bulb [66]. These results support the hypothesis that a functional uncoupling of the benzodiazepine recognition site from the GABA receptor in cortex, but not from the ion recognition site, may play a role in tolerance development. Changes in coupling may represent a common mechanism by which chronic benzodiazepine treatment can alter interactions at the macromolecular complex, resulting in benzodiazepine tolerance [66].

Considerations of the benzodiazepine deficiency syndrome must take into account the issue of benzodiazepine tolerance. Such tolerance is linked to losses in GABA-A receptor function and must be considered

with respect to results currently reported [67]. The benzodiazepines' hypnotic, anxiolytic, and anticonvulsant effects are revealed with development of tolerance after prolonged exposure to the drugs in both animals and humans. Losses in GABA-A receptor function have been linked to a substantial number of cases of benzodiazepine tolerance. The *in vivo* finding that benzodiazepine-evoked GABA-A receptor downregulation produced inconsistent results is significant [67]. Such variations have been attributed to differences in drug, treatment protocol, and tissue sampling after treatment.

Evidence is developing for neurosteroid regulation of GABA-A receptors *in vivo*. GABA has a role not only as an inhibitory neurotransmitter but also as a neurotrophic agent—namely, acting as a signal for neuronal growth, differentiation, and synapse formation [68].

GABA-A receptor subunits have been increased by the addition of NMDA to granule cell preparations in the cerebellum. This practice has led to speculation that the trophic actions of GABA and NMDA may share a common calcium-based mechanism. GABA-A-evoked upregulation of the GABA-A receptor observed in cultured cerebellum granule cells is contrasted with downregulations of cerebellar receptors observed *in vivo*. Possibly, the GABA-A receptors in granule cells experience upregulation *in vivo* but this process is masked by receptor downregulation occurring in other cerebellar cells (e.g., Purkinje cells) [69]. Also, endogenous GABA present in the granule cells in the embryonic cerebellum may be bathed in significant quantities of GABA, and the addition of GABA to cultures may restore this environment.

For tinnitus control treatment, both upregulation and downregulation are needed to activate and control the GABA-A receptors in brain. Chemical and metabolic labeling of GABA-A receptor polypeptides and tagging with high-affinity subunit-specific antibodies are planned in the future, to define and resolve remaining questions concerning up- and downregulation and use-dependent regulation of the GABA-A receptors in the tinnitus patient. Such investigations will provide answers to basic scientific questions about synthesis, assembly, and disassembly of GABA receptor subunits but also will enhance our understanding of the mechanisms behind GABA ligand-evoked actions in different clinical types and subtypes of predominantly central tinnitus.

7. *What are the regional localization effects of various benzodiazepines (e.g., Klonopin versus alprazolam or lorazepam)?* The data suggest that the effects of chronic benzodiazepine administration on the GABA-A receptor may be region-specific and receptor subtype-specific. Benzodiazepine receptors have been classified as types I and II [40]. Substantial neurochemical evidence exists to support the concept of multiple benzodi-

azepine receptors [70]. It is likely that several GABA-A receptors exist with different benzodiazepine-binding characteristics and that these subtypes have different regional specificities. Type I benzodiazepine receptors predominate in the cerebellum. The central benzodiazepine-binding site is the GABA-A-benzodiazepine receptor complex. This site is associated with the macromolecular complex that also binds to the major inhibitory neurotransmitter GABA (e.g., anticonvulsants, barbiturates, picrotoxin, and anxiolytic agents) [71].

At least two types of central benzodiazepine receptors with specific regional distribution are found in the mammalian brain. Type I receptors are concentrated in the cerebral cortex and cerebellum. Type II receptors are concentrated in the hippocampus, specifically the dentate gyrus and portions of the basal ganglia [72]. A functional pentameric GABA-A receptor is formed by coassembly of subunits selected from the family of 16 genes. Different subunits are responsible for sedative-motor effects, whereas other subtypes are primarily responsible for anxiolytic activity [40]. The affinities of different benzodiazepines for their receptors are considered significant if one is to understand the drugs' clinical pharmacology and their application for tinnitus therapy. Clinical and animal data suggest that the duration of pharmacodynamic action of benzodiazepines correlates with the affinity of these drugs for their specific binding sites.

Knowledge of both receptor binding affinities and pharmacokinetic properties of benzodiazepines is considered important in the rational clinical use of such drugs. This is particularly true during chronic therapy with benzodiazepines that have active metabolites with a higher or lower affinity for benzodiazepine-binding sites than does the parent compound. Changes in central-type GABA-A benzodiazepine receptors during 24-hour oral administration of alprazolam (2 mg/day) were measured using SPECT in nine healthy human subjects [73]. Clinical effects were altered 1–2 weeks after changes were noted in the receptor. It was concluded that the decrease of benzodiazepine receptor densities may be a major mechanism for tolerance development in humans. A small but significant decrease of receptor densities (10%) has been reported during chronic alprazolam administration. Receptors on the synaptic membrane and in vesicles cannot be differentiated with SPECT; therefore, if there was a transfer of the benzodiazepine receptors from synaptic membrane to vesicles (i.e., sequestration), the decrease of receptor densities measured in this study underestimated the decrease of the density on the cell membrane. Although a decrease of receptor densities and the development of tolerance were detected, the time courses of these events differed, and correlations between changes in the re-

ceptor during a state of tolerance in individual subjects were poor. These discrepancies indicate that the decrease of the receptor densities may be only one of several mechanisms involved in tolerance development. Animal studies have shown that low-dose alprazolam increased receptor densities [74] and motor activities [75], changes that were not detected with administration of other benzodiazepines [76].

## CONCLUSIONS

Preliminary results with  $^{123}\text{I}$  Iomazenil SPECT imaging of brain have revealed diminished benzodiazepine-binding sites in the medial temporal cortex in all six studied patients identified to have predominantly central, subjective, idiopathic, severe, disabling tinnitus. Diminished benzodiazepine-binding sites in the medial temporal cortex are considered consistent with the hypothesis implicating GABAergic mechanisms in the pathophysiology of tinnitus. A reduction in the number of benzodiazepine-binding sites in the medial temporal lobe cortex of tinnitus patients being studied supports the clinical recommendation for a trial of benzodiazepine therapy in attempting to control the affect component of tinnitus. Our study demonstrated, on a molecular level, a deficiency in GABAergic activity predominantly in the MTLs and cerebellum of brain in all six patients.

A benzodiazepine deficiency syndrome may exist in some tinnitus patients distinguished clinically by a severe, disabling tinnitus highlighted by the affect component—that is, patients complaining of alteration of behavior or emotion, stress, fear, anxiety, or depression. Preliminary results of this study recommend that a neurochemical or neuropharmacological approach toward tinnitus control include treatment directed at the GABA-A-benzodiazepine-chloride receptor. This benzodiazepine study provides a measurable parameter (i.e., the employment of  $^{123}\text{I}$  Iomazenil brain SPECT) that might aid the practitioner in customizing therapy for individual patients.

Finally, the finding of diminished benzodiazepine-binding sites in the medial temporal cortex of brain with  $^{123}\text{I}$  Iomazenil and SPECT imaging of brain is considered clinical support for the hypothesis of a final common pathway for tinnitus in the MTLs. For the first time in a predominantly central-type tinnitus patient, a neural substrate (i.e., the MTLs of brain) has been found to be deficient in GABA-A receptor density.

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