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^{125}I-iomazenil-benzodiazepine receptor binding during psychological stress in rats

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Objective: We investigated the changes in ^{125}I-iomazenil (^{125}I-IMZ) benzodiazepine receptor (BZR) binding with psychological stress in a rat model. ***Methods:*** Six male Wistar rats were placed under psychological stress for 1 hour by using a communication box. No physical stress was not received. 1.85 MBq of ^{125}I-IMZ was injected into the lateral tail vein and the rat was killed 3 hours later. Twenty-micrometer-thick sections of the brain were collected and % injected dose per body weight (%ID/BW) of eleven regions (frontal, parietal, temporal, occipital cortices, caudate putamen, accumubens nuclei, globus pallidus, amygdala, thalamus, hippocampus and hypothalamus) were calculated by autoradiography. The %ID/BW of rats which were placed under psychological stress was compared with that of 6 control rats. ***Results:*** The %ID/BW of rats which were placed under psychological stress diffusely tended to show a reduction in ^{125}I-IMZ-BZR binding. A significant decrease in BZR binding was observed in the hippocampus of the rats which were placed under psychological stress. ***Conclusion:*** ^{125}I-IMZ-BZR binding tended to decrease throughout the brain.

Key words: ^{125}I-iomazenil, benzodiazepine receptor, psychological stress, rat, communication box

INTRODUCTION

^{123}I-iomazenil (^{123}I-IMZ) is a radioligand that acts on central-type benzodiazepine receptors (BZR) as a partial inverse agonist.[1,2] ^{123}I-IMZ is thought to be involved not only in cerebrovascular diseases, but in neurological and psychological diseases as well. Some studies have presented clinical evaluations showing changes in BZR activity in patients with psychological diseases,[3–10] but no definite conclusions have been reached, and some points of controversy remain in both animal and human studies.

The present study used ^{125}I-IMZ to investigate the changes in BZR activity that occur during periods of psychological stress in a rat model.

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MATERIALS AND METHODS

Male Wistar rats weighting 260–315 g were used. Two or 3 rats were housed in plastic cages, and given food and water ad libitum. The rats were maintained on a 12-hour, light-dark cycle (light on from 08 : 00 to 20 : 00 hours) at a room temperature of 22–25°C and a relative humidity of approximately 45%.

A communication box, as shown in Figure 1, was used to establish differences in intraspecies emotional stimuli.[11,12] This box (32 cm × 32 cm × 39 cm) was equipped with a floor grid composed of 0.5 cm diameter stainless steel rods placed 1.3 cm apart. The box consisted of 4 small compartments (16 cm × 16 cm) divided by transparent plastic sheets. Plastic plates were placed on the grid floors of two compartments to prevent the rats from receiving an electronic shock. An electronic foot shock generator (MSG-001, TOYO SANGYOU, Japan) was used to produce a foot shock (3 mA) lasting for 10 seconds at intervals of 120 seconds. The rats were then divided into three groups as follows:

1. Physical stress group (foot-shock group; FS group)

which consisted of 6 rats placed in foot-shock compartments.

2. Psychological stress group (non-foot-shock group; NFS group) which consisted of 6 rats placed in non-foot-shock compartments. These rats did not receive any foot shocks but were exposed to various emotional stimuli from the foot-shock group.

3. Control group (CON group), which consisted of 6 rats placed under the same conditions as the NFS group on a different day. These rats did not receive either the foot shock or the psychological stress.

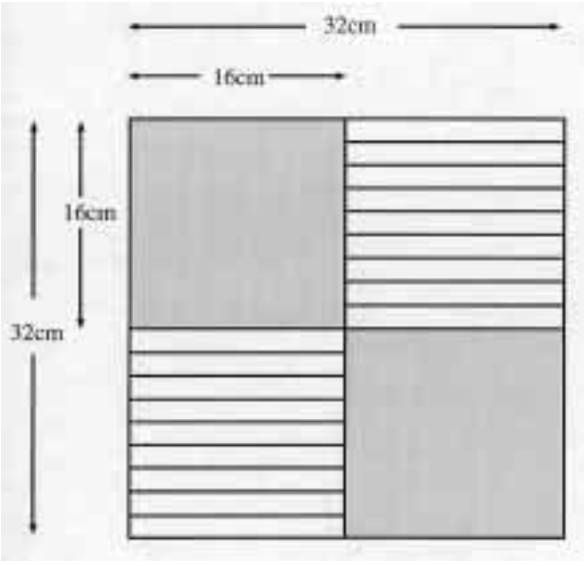

Fig. 1 Schema of the communication box. Foot-shock rats were placed individually in the two shaded area (foot-shock compartments). Psychological stress rats were placed in the two solid areas (non-foot-shock compartments). Foot-shock was delivered through grids of the floor in shaded areas. Solid areas were insulated by plastic plates.

The rats in the FS group were put individually into the compartments with an electric grid floor. The rats in the NFS group were put individually into the compartments that were insulated from an electric grid floor. The boxes were arranged so that the rats in the NFS group were surrounded by those in the FS group. During the stress session, the rats in the FS group were given intermittent electrical shocks delivered from an electrical shock generator through the grid floor for 1 hour. The rats in the CON group were put in a plastic box without foot shock or psychological stress. All treatments were performed between 12 : 30 and 13 : 30 hours.

At 13 : 40 hours, 1.85 MBq of ^{125}I-IMZ (Nihon Medi-Physics, Japan) was injected into the lateral tail vein of each rat in the NFS and CON groups. The rats were killed by decapitation at 16 : 40 hours (3 hours after the injection), and the brains were rapidly removed and frozen at −70°C in hexane and dry ice. Coronal sections of the brains were cut with a cryostat at −20°C.

Twenty-micrometer-thick sections were collected and dried for autoradiography. The sections were placed on imaging plates (BAS-MP, FUJI PHOTO FILM, Japan)

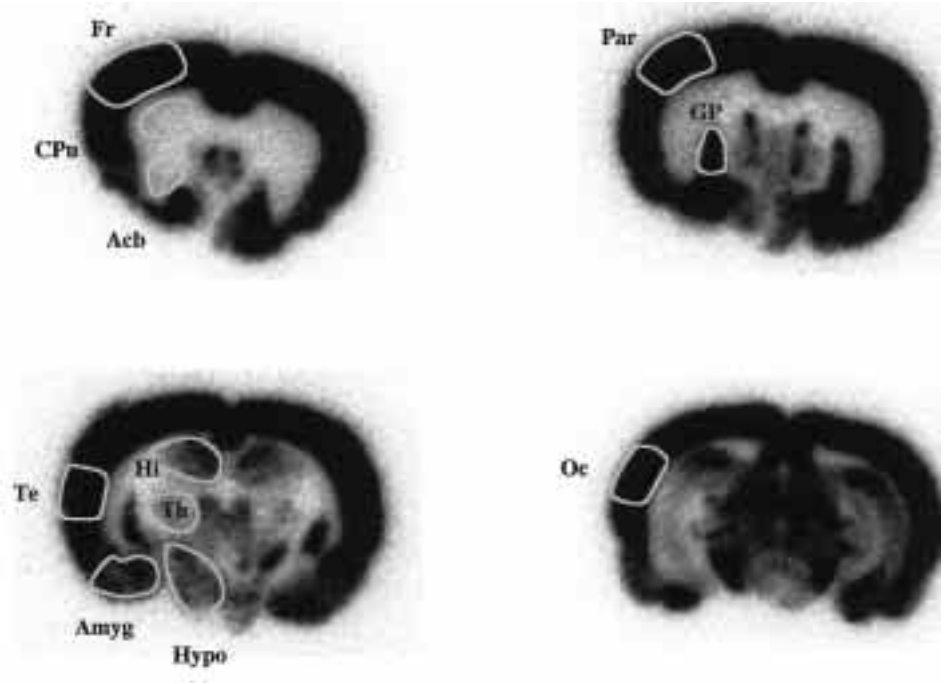

Fig. 2 Drawing of regions of interest (ROI) in four coronal sections of autoradiograms of the rat brain. Fr, frontal cortex; Cpu, caudate putamen; Acb, accumbens nucleus; Par, parietal cortex; GP, globus pallidus; Te, temporal cortex; Hi, hippocampus; Th, thalamus; Amyg, amygdala; Hypo, hypothalamus; Oc, occipital cortex.

and exposed for 10–14 days. The imaging plates were then removed and developed. The resulting autoradiograms were analyzed with a Macintosh computer-based image analysis system (Image Reader V1.4J).

Eleven regions of interest (ROIs; Fig. 2) were drawn on both sides of the brain, representing the frontal, parietal, temporal, occipital cortices, the caudate putamen, accumbens nuclei, globus pallidus, amygdala, thalamus, hippocampus and hypothalamus according to the Paxinos and Watson's atlas. Regional tracer binding was measured according to calibrated standards. Calibrated standards were expressed as the radiation dose (counts per minute) measured in a well-type counter per gram of polymer. Regional radiation doses per voxel were calculated according to calibrated standards. The % injected dose per body weight (%ID/BW) was calculated as follows: (regional radiation dose/voxel)/(injected radiation dose/kg of body weight) × 100 (%/kg).

The %ID/BW of the regions in the NFS and CON groups were calculated and compared. All results were analyzed by Mann-Whitney U analysis. In all cases, the criterion for significance was $p < 0.05$.

Table 1 The %ID/BW of each regions in NFS and CON groups

	%ID/BW (%/kg) (mean ± S.D.)	
	CON (n = 6)	NFS (n = 6)
frontal cortex	1.15 ± 0.37	0.88 ± 0.18
caudate putamen	0.21 ± 0.08	0.15 ± 0.02
accumbens nucleus	0.20 ± 0.08	0.14 ± 0.02
patrietal cortex	1.09 ± 0.32	0.88 ± 0.22
globus pallidus	0.72 ± 0.23	0.61 ± 0.09
temporal cortex	0.93 ± 0.28	0.70 ± 0.10
hippocampus	0.49 ± 0.12	0.33 ± 0.06*
thalamus	0.24 ± 0.06	0.18 ± 0.03
amygdala	0.51 ± 0.13	0.40 ± 0.06
hypothalamus	0.37 ± 0.09	0.29 ± 0.05
occipital cortex	0.67 ± 0.19	0.75 ± 0.12

*; $p < 0.05$

RESULTS

The results are shown in Table 1. The %ID/BW in the NFS group tended to show a reduction in ^{125}I-IMZ-BZR binding, compared to that in the CON group. A significant decrease in BZR was observed in the hippocampus in the NFS group (Fig. 3).

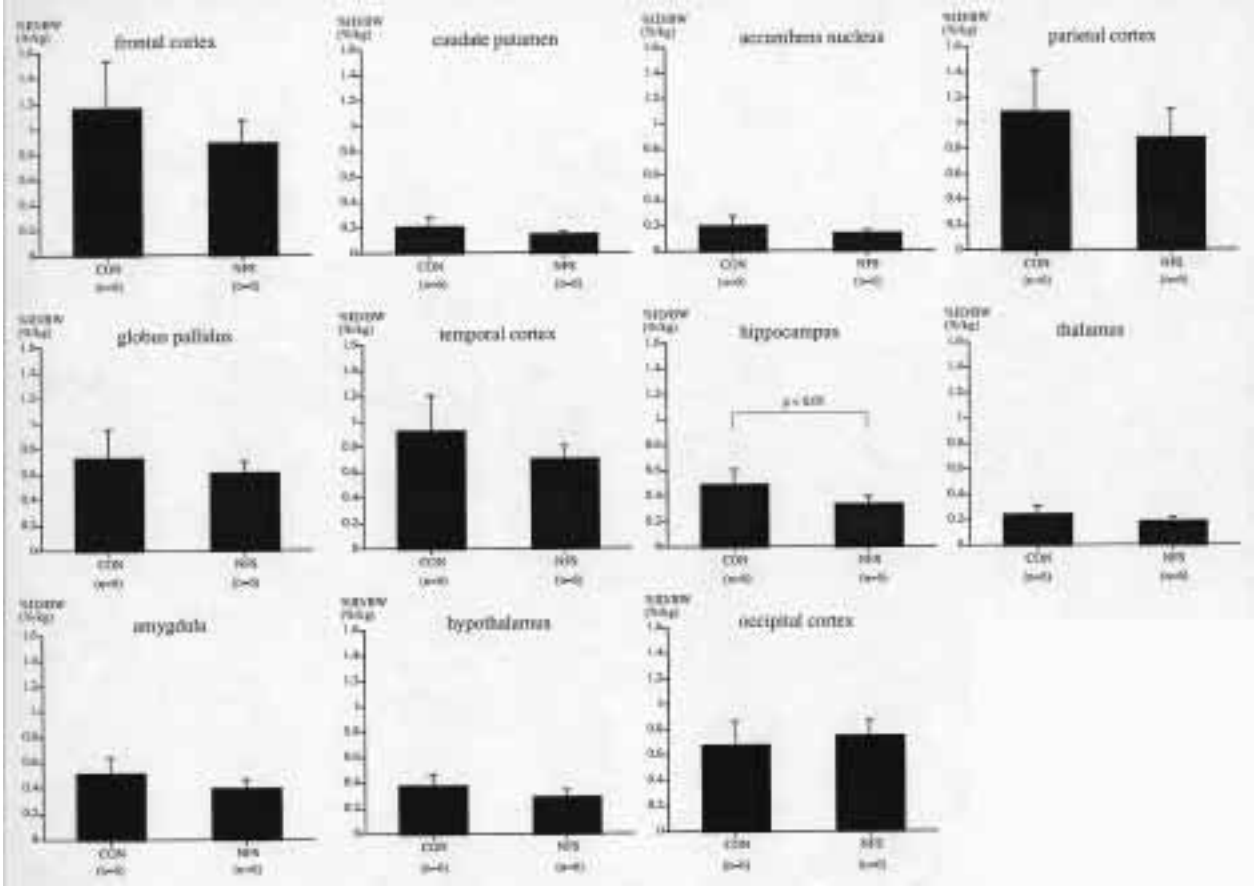

Fig. 3 The %ID/BW of each regions. The %ID/BW in the NFS group generally tended to show a reduction in ^{125}I-IMZ-BDZ receptor binding, compared to that of the CON group. A significant decrease in BDZ binding was observed in the hippocampus of the NFS group.

DISCUSSION

^{123}I-IMZ is a radioligand that acts on central-type BZR as a partial inverse agonist.[1,2] Central-type BZR is located on the alpha subunit of γ-aminobutyric acid ($GABA_A$) receptors, that forms chloride ionophores and is believed to be a major inhibitory neurontransmitter receptor in mammalian brains.[13,14]

Some studies have presented clinical evaluations showing changes in BZR activity in patients with psychological diseases,[3–10] but the methods used in assessment have varied from study to study, but no definite conclusion has been reached, because some points of controversy remain in both animal and human studies.

In animal studies, most of the subjects were subjected to swim stress tests,[6,8] but swim stress tests combine physical stress with psychological stress. We thought physical stress should be separated from psychological stress, and decided to use a communication box to solve this problem.

The communication box method, designed by Ogawa and Kuwahara, has been used to investigate behavioral and physiological changes caused by psychological stress.[11,12,15–21] The important feature of this method is that an animal which is exposed to physical stress such as a foot shock can induce psychological stress in another animal by means of intraspecies emotional communication (i.e., the emotional response of the NFS group upon observing signs of physical stress, such as struggling, vocalizing, defecating, urinating and jumping, in the FS group).

The methods used in previous human studies have been controversial because studies on a simplified model were difficult: some patients had received long-term medication prior to the study, similar diseases were confused or combined, and the amount of stress experienced by the subjects varied.[3–5,7,10]

We examined the changes in ^{125}I-IMZ-BZR binding during periods of psychological stress in a simplified animal model. As shown in Figure 3, the binding of ^{125}I-IMZ to BZR generally tended to decrease.

We believe that stress-induced endogenous agents have a high affinity for BZR,[22] and that ^{125}I-IMZ binding to BZR is BZR occupancy by endogenous benzodiazepine inverse agonists. In view of the finding that ^{125}I-IMZ-BZR binding tended to decrease throughout the brain, endogenous benzodiazepine inverse agonists are likely to be diffusely secreted and act throughout the entire brain. It is disputable whether stress-induced endogenous agents have a high affinity for BZR constantly or transiently.

We intend to continue this study by investigating the correlation between ^{125}I-IMZ-BZR binding activity and the amount of psychological stress, time progress and the effect of treatment.

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REFFERENCES

1. Beer HF, Blauenstein PA, Hasler PH, Delaloye B, Riccabona G, Bangerl I, et al. *In vitro* and *in vivo* evaluation of iodine-123-Ro 16-0154: a new imaging agent for SPECT investigations of benzodiazepine receptors. *J Nucl Med* 1990; 31: 1007–1014.
2. Holl K, Deisenhammer E, Dauth J, Carmann H, Schubiger PA. Imaging benzodiazepine receptors in the human brain by single photon emission computed tomography (SPECT). *Nucl Med Biol* 1989; 16: 759–763.
3. Uchiyama M, Sue H, Fukumitsu N, Mori Y, Kawakami K. Assessment of cerebral benzodiazepin receptor distribution in anxiety disorders by ^{123}I-iomazenil-SPECT: comparison to cerebral perfusion scintigraphy by ^{123}I-IMP. *NIPPON ACTA RADIOLOGICA* 1997; 57: 41–46.
4. Nakabeppu Y, Nakajo M, Naruo T, Obo Y, Nozoe S, Takigawa M, et al. I-123 iomazenil SPECT in patients with mental disorders. *Ann Nucl Med* 1997; 11: 339–344.
5. Tokunaga M, Ida I, Higuchi T, Mikuni M. Alterations of benzodiazepine receptor binding potential in anxiety and somatoform disorders measured by ^{123}I-iomazenil SPECT. *Radiation Medicine* 1997; 15: 163–169.
6. Takahashi M, Odano I, Fujita S, Ohkubo M. ^{125}I-iomazenil binding shows stress- and/or diazepam-induced reductions in mouse brain: supporting data for ^{123}I-iomazenil SPECT study of anxiety disorders. *Ann Nucl Med* 1997; 11: 243–250.
7. Torizuka K, Uemura K, Toru M, Shinohara Y, Nishimura T, Yonekura Y, et al. A phase 3 clinical trial of ^{123}I-iomazenil, a new central-type benzodiazepine receptor imaging agent (part 2)—report on clinical usefulness in diagnosis of degenerative neurological diseases and mental disorders—. *KAKU IGAKU (Jpn J Nucl Med)* 1996; 33: 303–318.
8. Weizman R, Weizman A, Kook KA, Vocci F, Deutsch SI, Paul SM. Repeated swim stress alters brain benzodiazepine receptors measured *in vivo*. *J Pharmacol Exp Ther* 1989; 249: 701–707.
9. Kaneko K, Kurumaji A, Shibuya H, Nabeshima T, Toru M. The effects of single and repeated phencyclidine administration on [^{125}I] iomazenil binding in the rat brain. *Nerochem* 1996; 29: 279–287.
10. Northoff G, Steinke R, Czcervenka C, Krause R, Ulrich S, Danos P, et al. Decreased density of GABA-A receptors in the left sensorimotor cortex in akinetic catatonia: investigation of *in vivo* benzodiazepine receptor binding. *J Neural Neurosurg Psychiatry* 1999; 67: 445–450.
11. Ishikawa M, Hara C, Ohdo S, Ogawa N. Plasama corticosterone response of rats with sociopsychological stress in the communication box. *Physiol Behav* 1992; 52: 475–480.
12. Ogawa N, Kuwahara K. Psychophysiology of emotion-communication of emotion. *Jpn J Psychosom Med* 1966; 6: 352–357.

13. Hommer DW, Skolnick P, Paul SM. The benzodiazepine/GABA receptor complex and anxiety. In *Psychopharmacology*, 3rd eds (Meltzer HY ed; Raven Press, New York), 1987: 977–983.
14. Moehler H, Okada R. Benzodiazepine receptor: demonstration in the central nervous system. *Science* 1977; 198: 849–851.
15. Nomura N, Maeda N, Kuratani K, Yamaguchi I. Sulpiride specifically attenuates psychological stress-induced gastric lesions in rodents. *Jpn J Pharmacol* 1995; 68: 33–39.
16. Hotta M, Shibasaki T, Arai K, Demura H. Corticotropin-releasing factor receptor type 1 mediates emotional stress-induced inhibition of food intake and behavioral changes in rats. *Brain Res* 1999; 823: 221–225.
17. Musty RE, Jordan MP, Lenox RH. Criterion for learned helplessness in the rat: a redefinition. *Pharmacol Biochem Behav* 1990; 36: 739–744.
18. Nomura K, Maeda N, Yoshino T, Yamaguchi I. A mechanism of 5-HT_3 receptor mediation is involved etiologically in the psychological stress lesion in the stomach of the mouse. *J Pharmacol Exp Ther* 1994; 271: 100–106.
19. Zhukov DA, Vinogradova EP. Agonistic behavior during stress prevents the development of learned helplessness in rats. *Neuroscience and Behavioral Physiology* 1998; 2: 206–210.
20. Ishikawa M, Ohdo S, Watanabe H, Hara C, Ogawa N. Alteration in circadian rhythm of plasma corticosterone in rats following sociopsychological stress induced by communication box. *Physiol Behav* 1995; 57: 41–47.
21. Sato Y, Suzuki N, Horita H, Wada H, Shibuya A, Adachi H, et al. Effects of long-term psychological stress on sexual behavior and brain catecholamine levels. *J Androl* 1996; 17: 83–90.
22. Biggio G, Concas A, Serra M, Salis M, Corda MG, Nurchi V, et al. Stress and β-carbolines decrease the density of low affinity GABA binding sites; an effect reversed by diazepam. *Brain Res* 1984; 305: 13–18.