Original Article

Writing errors in ALS related to loss of neuronal integrity in the anterior cingulate gyrus

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Abstract

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by loss of motor neuron and various cognitive deficits including writing errors. ^{11}C-flumazenil (FMZ), the positron emission tomography (PET) $GABA_A$ receptor ligand, is a marker of cortical dysfunction. The objective of this study was to investigate the relationship between cognitive deficits and loss of neuronal integrity in ALS patients using ^{11}C-FMZ PET. Ten patients with ALS underwent both neuropsychological tests and ^{11}C-FMZ-PET. The binding potential (BP) of FMZ was calculated from ^{11}C-FMZ PET images. There were no significant correlations between the BP and most test scores except for the writing error index (WEI), which was measured by the modified Western Aphasia Battery – IVB (WAB –IVB) test. The severity of writing error was associated with loss of neuronal integrity in the bilateral anterior cingulate gyrus with mild right predominance (n=9; x = 4mm, y = 36mm, z = 4mm, Z=5.1). The results showed that writing errors in our patients with ALS were related to dysfunction in the anterior cingulate gyrus.

Key words; Amyotrophic lateral sclerosis, writing error, cognitive dysfunction, PET, anterior cingulate gyrus

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disorder characterized by progressive muscular atrophy and weakness with upper motor neuron impairment. It has traditionally been believed that intelligence is spared except in a small proportion of patients. Mitsuyama [1] described patients with dementia as having memory difficulty, global intellectual impairment, personality change, emotional disorder and loss of spontaneous speech. These deficits are consistent with frontotemporal lobar degeneration (FTLD).

Recent studies have shed light on the mild cognitive impairment of ALS patients who do not meet the criteria of FTLD [2]. The published rate of ALS patients with cognitive impairment varies from 3% to 52% [3-6]. As a result of intensive explorations of frontal lobe function, impairment of verbal fluency and executive function have been identified as specific deficits in ALS [4, 7-11]. Some Japanese investigators described that ALS patients frequently had agraphia without aphasia regardless of whether they had dementia [12-15]. Omission of a Japanese *kana* letter was the most frequent error, and substitution, displacement, and incorrect letters were also often observed. The pattern of errors looked like they occurred from a frontal lobe lesion, which was supported by the finding of a reduction in cerebral blood flow in the frontal lobe in a single photon emission computed tomography (SPECT) study of a few cases. An autopsied case with progressive agraphia and ALS-D showed marked degeneration of the left middle frontal gyrus including Exner's area [16]. Thus,

dysfunction of the frontal lobe was suspected as being responsible for the writing error in patients with ALS; however, because the number of reported cases was small, the pathological locus has not been conclusively identified.

The $GABA_A$ receptor ligand ^{11}C-flumazenil (FMZ) is a probe used in positron emission tomography (PET) for the central-type benzodiazepine receptor (BZR) [17]. In vitro studies of specimens obtained from patients with epileptogenic brain lesions demonstrated altered GABAergic neurotransmission in the perilesional epileptic cortex [18, 19]. Therefore, FMZ PET is a sensitive, noninvasive method for visualizing focal cortical dysfunction that may represent epileptogenic zones. Because GABA receptors are abundant in the cortex, cerebral BZRs co-located with GABA-A receptors can also be used as a marker of neuronal viability [20].

In this study, we performed several cognitive screening tests in non-demented ALS patients and identified the locus of their cognitive symptoms using FMZ PET.

Subjects and Methods

Subjects

Ten patients with sporadic ALS agreed to participate in this study from September 2007 to June 2008. At the time of the examination, the patients were in the following categories of the revised El Escorial Criteria: 2 patients definite, 4 probable, 3 possible, and 1 suspected. All patients deteriorated in the following year, developing

probable or definite ALS by 2010 [21]. The lesion at onset was located in the brainstem in 7 cases, cervical region in 2 cases and lumbosacral region in 1 case. Table 1 shows characteristics of the patients. No patient had respiratory symptom, and no patient had highly impaired forced vital capacity (%FVC >70). One patient had encephalitis several years prior without any sequelae while her score on the Raven Progressive Colored Matrices (RPCM) was lower than -2SD. Another patient was left-handed. Image analysis was performed within one month after having administered the neuropsychological examinations.

All patients gave written informed consent according to the Helsinki Declaration. This study was approved by the Ethics Committee of Hokkaido University Graduate School of Medicine.

Neuropsychological examination

Since we speculated that patients with ALS tended to have writing errors as a result of frontal lobe disorder, we mainly examined their ability of writing and frontal lobe function. A comprehensive test battery was developed as shown in Table 2. All ten patients underwent the neuropsychological tests. To confirm general cognition, all subjects were administered the Japanese Raven's colored progressive matrices test (RCPM). One of the ten patients could not perform the writing and trail-making tests at all because of disability of the dominant hand. Another patient could not perform the trail-making test because of fatigue. In addition, two patients with

severe bulbar sign could not perform the verbal repetition test.

To quantify the writing errors, we used the original writing error index (WEI), which we had developed in our previous study [15]. Briefly, the patients were shown a picture of people at a picnic and were requested to give a written description of the photograph. The WEI was calculated as follows:

WEI = (number of errors / total number of written words) x 100.

Errors include omissions, replacements, spelling rearrangements, nonexistent letters (ideogram, or *kana*) and nonexistent characters (morphograms, or *Kanji*). An incomprehensible or grammatically strange sentence was considered to be one error.

^{11}C-flumazenil positron emission tomography

PET was performed using the ECAT EXACT HR + scanner (Asahi-Siemens, Tokyo, Japan) with in-plane and axial resolutions of 4.5 mm and 3.71 mm, respectively. Photon attenuation was corrected with a 5 min transmission scan. FMZ PET procedures were performed as previously described [22].

Dynamic FMZ PET scans were acquired in all patients. Drugs that affect BZR were withdrawn at least one week before the FMZ PET studies. The injected dose of FMZ was 370 MBq in each patient. A set of 27 sequential PET frames of increasing duration were obtained over 60 minutes after FMZ injection, according to the following

protocol: 40 sec x 1 frame, 20 sec x 10 frames, 60 sec x 4 frames, 180 sec x 4 frames and 300 sec x 8 frames. A reference tissue compartment model was used for noninvasive estimation of binding potential (BP) with a time-activity curve in the pons as an indirect input function [22]. The equations that we used were previously described [22]. BP was estimated by the nonlinear least squares method using the equations for the reference tissue compartment model [23].

MRI

MRI was performed using a 1.5 Tesla scanner (Magnetome Vision or Magnetome Symphony, Asahi-Siemens, Tokyo, Japan). Three-dimensional T1-weighted images were acquired with the magnetization-prepared rapid gradient-echo (MP-RAGE) sequence. Transaxial T2 and T2*-weighted images and FLAIR images were acquired. All images were acquired with 5 mm slice thickness and no slice gap. Coronal and sagittal images were obtained in some cases.

Image Analysis

Image analysis was performed using statistical parametric mapping (SPM2 and SPM5 softwares) in Matlab version 7.6 (Mathworks, Natick, MA, USA) on a Microsoft Windows-based workstation [24]. The ^{11}C-flumazenil binding potential images (FMZ BP) were first co-registered with the three-dimensional MR images by the mutual information technique. The three-dimensional MR images were spatially normalized using T1-weighted imaging templates included in the SPM package. The FMZ BPs were spatially normalized with the same parameters used to spatially normalize the MRI. The spatially normalized FMZ BPs were smoothed with an isotropic Gaussian kernel of 8 mm. Global counts were estimated using proportional scaling. The threshold value was set at 0.80.

We performed a separate linear regression for each voxel, in which the normalized BP image voxel in each scan was the dependent variable and each WEI score was the independent variable, as described previously [25].

Statistics across the entire brain are displayed as *Z*-scores. Results with Family Wise Error-corrected *P*-values < 0.05 and extent threshold = 20 voxels are also shown.

Results

The neuropsychological test scores are shown in Table 2. As reported in a previous study, patients with ALS performed poorly at picture arrangements and writing (the average WEI of the patients was 6.0 while that of 16 normal controls was 2.9) [15]. In the present study, there were no significant correlations between BP and scores except for the WEI. Various regions showed a correlation between BP and the WEI including the bilateral anterior cingulate gyrus (e.g., bilateral middle frontal gyrus, bilateral claustrum, bilateral middle temporal gyrus, bilateral parahippocampal gyrus, left inferior frontal gyrus, and left inferior temporal gyrus) under Family Wise Error-corrected $P < 0.05$ (Figure 1). Under Family Wise Error-corrected $P < 0.01$, the bilateral anterior cingulate gyrus with mild right predominance (n=9; x = 4 mm, y = 36 mm, z = 4 mm, Z=5.1) was the only region identified (Figure 2). Figure 3 shows that there was a negative correlation between the BP at coordinate x = 4 mm, y = 36 mm, z = 4 mm and the WEI (n=9; family-wise error corrected *P*-value < 0.01; extent threshold = 20 voxels). There was no obvious difference between analyses performed using SPM 2 or SPM5.

Discussion

^{11}C-flumazenil PET studies in ALS patients by Wicks et al. [1] revealed that the binding potential of ^{11}C-FMZ in the inferior frontal gyrus, superior temporal gyrus, and anterior insula was decreased in patients with impaired verbal fluency and that the score in a confrontation naming test was correlated with the binding potential in the left middle frontal gyrus and left cuneus. Turner et al. [11,26] reported that ALS patients had decreased binding potential in the left frontotemporal junction and anterior cingulate gyrus. However, there has been no information published to date about the association of agraphia with the anterior cingulate gyrus.

Previous studies identified impairment of the anterior cingulate gyrus in patients with ALS [8, 26-28]. There is also evidence that the anterior cingulate gyrus is involved with dysgraphia: the evidence includes neurohistopathological findings of neuronal loss, a reduced circulation rate as determined by PET, and a decrease in the NAA/Cr ratio as evaluated by ^{1}H-MRS, especially in patients with bulbar palsy [27, 28]. Our results differ from previous studies that suggested involvement of the dorsal cingulate gyrus in

Brodmann area 32 or the posterior region of the anterior cingulate gyrus with occasional left-dominant hemisphere lesions [26, 27]. Furthermore, conflicting observations exist on the dominance of the right versus left hemisphere of the anterior cingulate gyrus. Abrahams et al. reported that impairment of the right dominant anterior cingulate gyrus causes deficits on tests of letter fluency [8].

Besides the laterality of the relevant hemispheric lesion, the pathogenic role of the anterior cingulate gyrus in the agraphic impairment of ALS patients has yet to be defined. A few cases in a SPECT study suggested a frontal lobe lesion, but their spatial resolution was restricted [13]. Only one case autopsy case showed degeneration of Exner's area [16]. There is no direct evidence that links the writing center and anterior cingulate gyrus. The anterior cingulate gyrus plays a key role in attention behavior, particularly the executive control of actions such as detecting errors [28-31]. Electroencephalographic studies reveal that error-related negativity (ERN) is produced in the anterior cingulate gyrus in response to errors and is followed 150 – 400 ms later by production of error positivity (PE) [31]. These findings suggest that the agraphia associated with anterior cingulate gyrus lesions in ALS patients is potentially due to

deficits in attention. However, strictly speaking, the relevant region described in the literature as Brodmann area 32 differs from the region in our study which was Brodmann area 24 [32]. As reported previously, features of the writing errors of patients with ALS included omission, substitution, displacement, and inappropriate placement of the phonic marks of *kana* [13, 14]. These could occur in a patient with both attention deficit and pure agraphia. In our previous study, other cognitive examinations were administered to patients with ALS [15]. Although digit span, which was considered to reflect attention, was preserved in patients who had writing errors, a problem with attention may be a strong candidate for the pathology of writing errors in patients with ALS, but further studies are necessary to reach a conclusion.

In view of the network of connections between the anterior cingulate gyrus and the basal ganglia, cerebral dysfunction other than attention deficits may also contribute to agraphia in ALS patients [33]. Clinical analyses of central nervous system diseases have shown impairment of the anterior cingulate gyrus as a putative cause of apathy and akinetic mutism [33]. ALS patients with clinically diagnosed bulbar palsy are reported to have limited speech abilities [34]. These conditions may be attributed to

fronto-temporal lobe degeneration, but a possible association between apathy and akinetics with agraphia still needs to be further investigated.

In this study, it is possible that the strict statistical condition masked the true lesion. Indeed, when statistical significance was set at *P*-values < 0.05 without correction, numerous areas were associated with writing error (data not shown). Among them, the left middle frontal gyrus was noteworthy. A larger number of samples is necessary to confirm the results, and the study is ongoing.

Isolated vascular disorders in the anterior cingulate gyrus are so rare that its function is not fully understood. Clinical analyses using ^{11}C-FMZ PET may facilitate understanding of the physiology of the anterior cingulate gyrus as well as its pathogenetic role in the agraphia seen in patients with ALS.

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Figure legends

Figure 1. Brain images of a separate linear regression (FWE corrected P-value < 0.05)

Brain regions with significant negative correlations between FMZ binding potential and error rate on the writing test (WAB-IV B) (family-wise error corrected *P*-value < 0.05 extent threshold = 20voxels), are shown.

Figure 2. Brain images of a separate linear regression (FWE corrected P-value < 0.01)

Brain regions with significant negative correlations between FMZ binding potential and error rate on the writing test (WAB-IV B) (family-wise error corrected *P*-value < 0.01 extent threshold = 20voxels) are shown. The region is the bilateral anterior cingulate gyrus with mild right dominance.

Figure 3. Statistical parametric mapping analysis

The normalized voxel binding potential rate at coordinates x = 4 mm, y = 36 mm, z = 4mm was significantly correlated with the error rate on the writing test (WAB-IV B) (family-wise error corrected *P*-value < 0.01 extent threshold = 20voxels).

Table 1. Clinical features among the 10 ALS patients

Total number of patients	10
Male / female ratio	6:4
Age (years, mean ± SD)	61.1 ± 11.7
Months from onset (mean ± SD)	19.1 ± 5.1
ALS-FRS-R (total) (mean ± SD)	35.6 ± 8.4
ALS-FRS-R (1-3) (mean ± SD)	7.0 ± 4.6

ALS-FRS-R: ALS functional rating scale revised, *SD*: standard deviation

Table 2. Neuropsychological test scores

Test	**n**	**mean ± SD**	**FS**
General intelligence			
Raven's colored progressive matrix test (RCPM)	10	29.8 ± 44	37
Picture arrangement (WAIS-R IV-3, 4, 5)	10	1.6 ± 1.1*	3
Attention			
Trail-making tests A (TMT-A)	8	59.7 ± 35.3s	
Trail-making tests B (TMT-B)	8	154.9 ± 71.8s	
(TMT-B) - (TMT-A)	8	84.4 ± 46.4s	
Frontal assessment battery: sensitivity to interference and inhibitory control			
FAB-4: conflicting instruction	10	2.6 ± 1.0	3
FAB-5: go/no-go test	10	2.1 ± 1.2	3
Verbal function			
Repetition (WAB-III)	8	98.6 ± 1.9	100
Composition, writing error index (WEI**)	9	6.0 ± 0.1***	0

* The result in healthy controls was 2.9±0.4 (n = 18) [15]. $p<0.05$ vs. healthy controls.

** The WEI was calculated as described previously [15].

***The result in healthy controls was 2.3±3.0 (n = 14) [15]. $P<0.05$ vs. healthy controls.

SD: standard deviation, FS: full score, n: number of patients, s: seconds

Fig.1

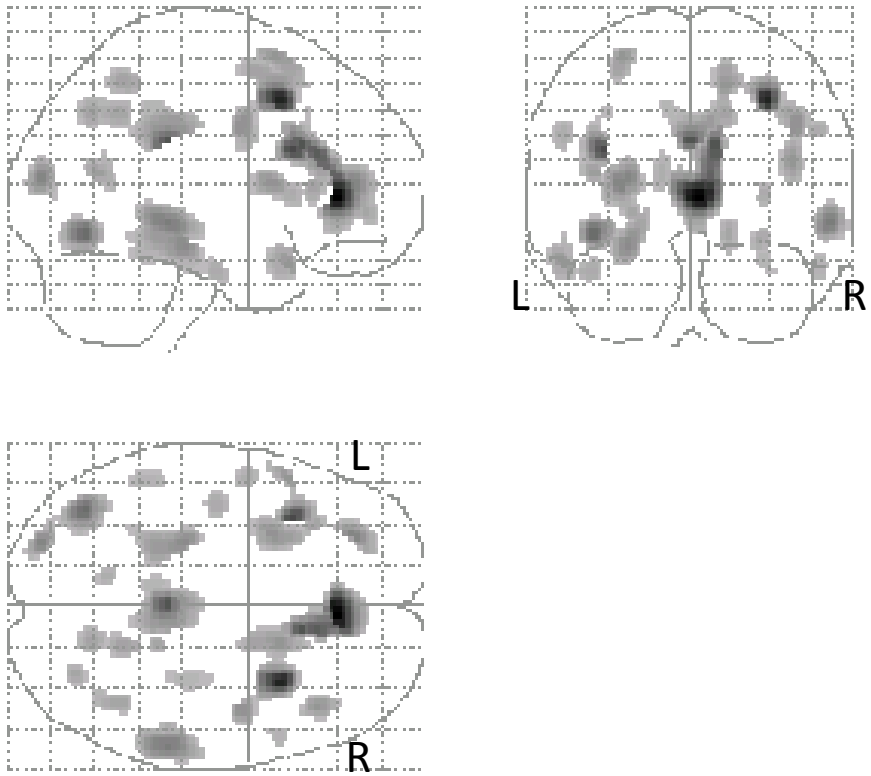


Fig.2

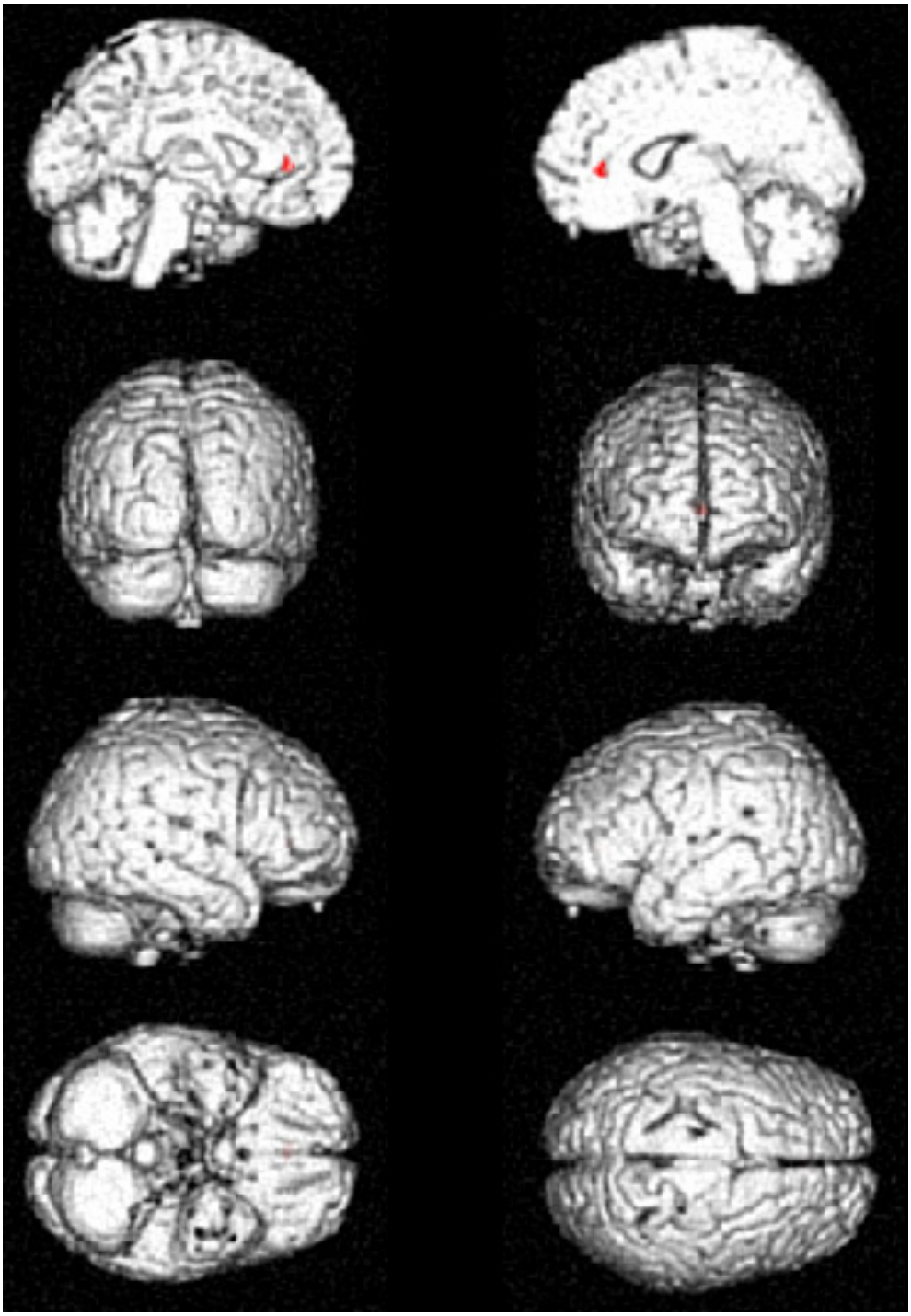
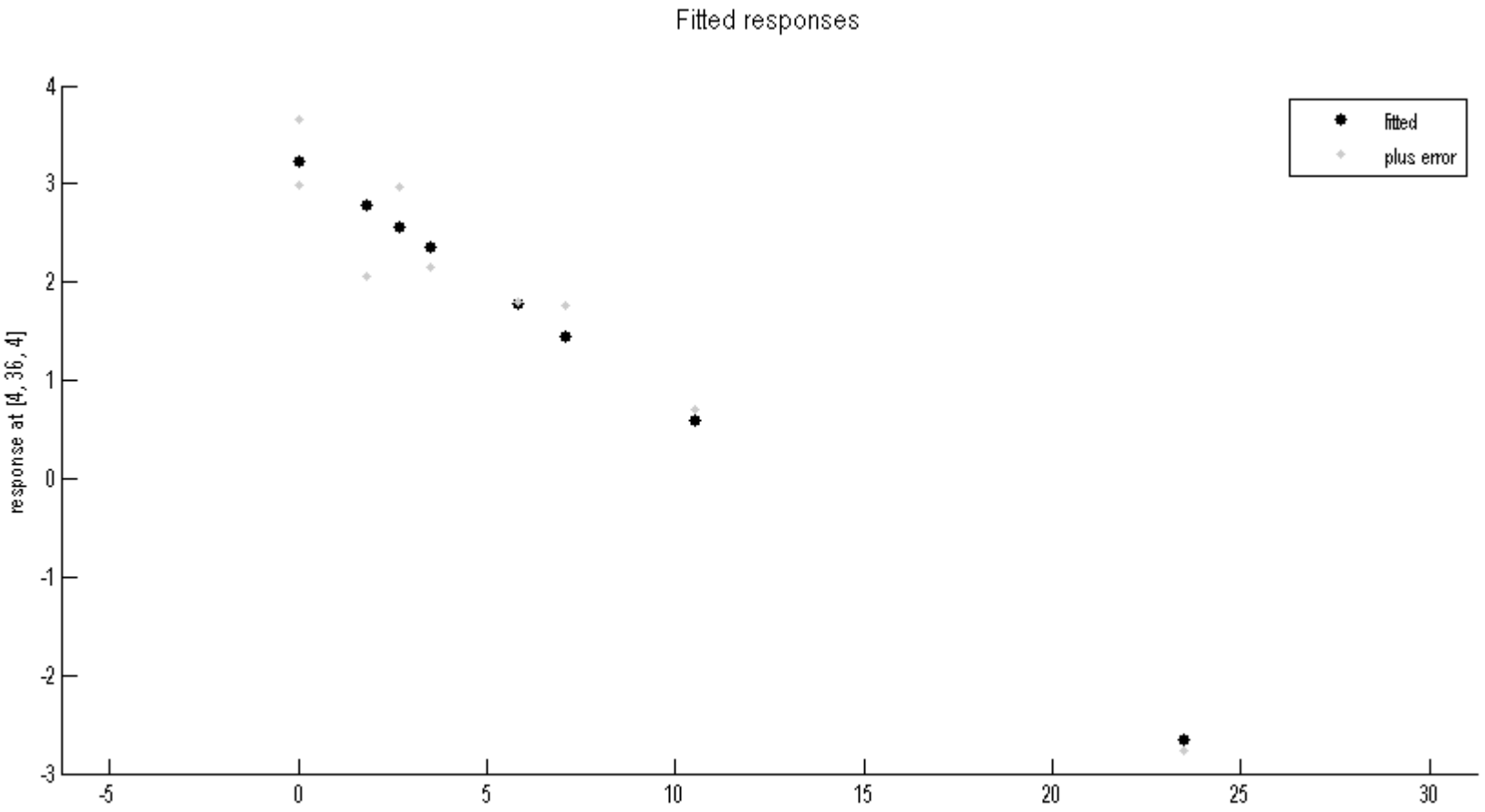


Fig.3



the error rate on the writing test