The cerebral correlates of different types of perseveration in the Wisconsin Card Sorting Test

Y Nagahama, T Okina, N Suzuki, H Nabatame, M Matsuda

Objectives: To explore the neural substrates corresponding to the perseverative errors in the Wisconsin Card Sorting Test (WCST).

Methods: The study examined the correlations between the WCST performances and the SPECT measurements of regional cerebral blood flow (rCBF) in subjects with neurodegenerative dementia. Negative non-linear correlations between the rCBF and the two different types of the perseverative errors (''stuck-in-set'' and ''recurrent'' perseverative errors) were calculated on a voxel basis and volume-of-interest basis in the mixed groups of 72 elderly and dementia patients.

Results: The stuck-in-set perseverative error was associated with the reduced rCBF in the rostrodorsal prefrontal cortex, whereas the recurrent perseverative error was related to the left parietal activity but not to the prefrontal activity.

Conclusions: These findings augment evidence that the rostrodorsal prefrontal cortex crucially mediates attentional set shifting, and suggest that the stuck-in-set perseverative errors would be a true pathognomonic sign of frontal dysfunction. Moreover, this study shows that the recurrent perseverative errors may not be associated closely with the prefrontal function, suggesting that this error and the stuck-in-set error should be differentially estimated in the WCST.
To explore the neural substrates corresponding to the particular perseverative errors in the WCST, we planned to examine the correlations between the WCST performances and the resting regional cerebral blood flow (rCBF) in patients with neurodegenerative dementia. Previous neuroimaging studies have shown that analysis of the correlations between cognitive performance and resting cerebral metabolism and blood flow is sensitive to unravel the neural substrates of cognitive impairments. Most previous studies that examined the relation between the behavioural parameters and the neural activity employed a simple linear function as the regressor. However, the linear function may not be good enough to characterise brain regions by their rCBF value in relation to the parameters. There is now considerable evidence to suggest that ageing and disease processes in the brain introduce compensatory mechanisms to resist the “to be reduced” cognitive performances. Recent neuroimaging studies demonstrated that in elderly people and early Alzheimer’s disease (AD) patients the neural activity in several brain regions, such as prefrontal and hippocampal cortex, shows greater increase than that in young patients during cognitive tasks, despite the patients’ task performance being poorer than the healthy subjects. Furthermore, a histochemical study showed that the choline acetyltransferase activity in the frontal and hippocampal cortex was used regulated in subjects with mild cognitive impairment (MCI) and these activities were actually reduced in the clinically evident AD. These physiological and histochemical findings suggest that during early cognitive decline neural activity in these brain regions could be reserved to compensate for degenerative processes and may protect these individuals from becoming obviously demented. We can therefore hypothesise that in the parametric regression analysis the relation between the rCBF and the reduced task performances might be non-linear; during the performance reduction is mild, the neural activity—that is, rCBF—in the corresponding brain regions is preserved because of a compensatory mechanism and then the rCBF is decreased as the task performance is further impaired.

In general, how good the regression fits depends on the type of the basis function chosen. As the relation between parameters and rCBF could be non-linear, the a priori definition of a fit function as a linear one might lead to an insufficient or partial result. Thus, we modelled the rCBF changes with a non-linear function—for example, a second order polynomial. We expected that the use of the non-linear basis function in conjunction with the general linear model facilitates the detection of rCBF changes in brain regions that might not have been so evident using simple linear regression.

SUBJECTS

Seventy two patients were included in the study: six elderly subjects (54–86 years), 12 patients with MCI (60–79 years), three patients with frontotemporal lobar degeneration (FTD, 60–76 years), and 51 patients with probable AD (66–85 years). All were outpatients who attended the Memory Clinic, Shiga Medical Center between July 2000 and March 2003.

Patients with AD fulfilled the DSM III-R criteria for “primary degenerative dementia” and NINCDS/ADRDA criteria for probable AD. Patients with amnestic MCI were diagnosed according to recommendation criteria of the American Academy of Neurology. Patients with frontotemporal lobar degeneration were diagnosed using the clinical diagnostic criteria for FTD. All patients were interviewed and received a comprehensive neurological examination prior to inclusion in the study. Exclusion criteria included a history of stroke, psychiatric disorder, significant head trauma, alcohol abuse, or evidence of other systemic or neurological disorders that may compromise cognition. All patients received the Mini-Mental State Examination (MMSE) and severely demented patients (whose MMSE score was less than 13) were excluded from the study. All patients were scanned with head computed tomography (CT) or MRI, and no abnormalities were found other than atrophy and/or mild periventricular low densities. Most patients received no psychotropic medication in the month preceding the study except for 11 patients: seven AD were medicated with donepezil, two AD with donepezil and tiapride, one AD with paroxetine, one FTD with maprotiline. All the neuropsychological tests and the neuroimaging were parts of routine clinical examinations, and informed consent was obtained from all patients and/or caregivers concerning the nature and purpose of the procedures.

The WCST

A computerised, modified version of the WCST was administered to all the patients. In the WCST, the patients were instructed to match a “response” card to one of the four “stimulus” cards on the basis of one of the three possible categories of number, colour, or shape, by clicking one of the stimulus cards using a mouse. In some patients who were unfamiliar with using a computer mouse, the patients selected their answers by pointing at one of the stimulus cards with their index finger and the examiner clicked them. As in the Nelson’s version, we used the 48 (2×24) response cards that share one and only one attribute with the stimulus cards. Patients were not informed of the correct sorting principle, nor were they told when the principle would shift during the test, but they were informed of the three possible categories before testing. The patients were required to determine which one was correct based solely on the feedback that indicated whether each response was “correct” or “wrong”. When the patient maintained a correct progression through six trials, the rule was changed without warning and they had to shift the sorting rule from the previously relevant category to the other one to yield correct answers. Unlike the common forms of the traditional WCST in which the test ends after six correct categories were achieved, the testing in this study continued until all 48 cards were sorted.

The responses were scored according to Heaton’s criteria but with one minor exception: the first unambiguous error repeating the previously correct principle was not scored as perseveration because the subject could not receive feedback indicating that the previously correct principle was now incorrect. We first calculated the raw scores of Heaton’s perseverative errors and non-perseverative errors. Heaton’s perseverative errors include the following three criteria:

- Incorrect responses that have been correct in the previous stage.
- In cases of patients who were so perseverative that they never attained the first category, the first incorrect unambiguous response in stage one was regarded as the “perseverated to” criterion.
- If the subject made three unambiguous incorrect responses in succession according to another principle—that is, the principle that was neither the correct one in the current stage nor the one that was defined as the perseverated to principle according to the rules given above—the perseverated to criterion was changed to that principle.

Then we divided the perseverative errors into two different perseverative scores: the “stuck-in-set” perseverative error (SIS-PE) and the “recurrent” perseverative error (Rec-PE). The SIS-PE is similar to the perseverative errors
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defined by Nelson: exact repetitions of the immediately preceding incorrect response. It reflects a “short range” perseveration in which the patients could not shift their responding even when they knew the rule was wrong—that is, the failure to shift attentional set. Rec-PE is the inappropriate repetition of a previous response after an intervening response, in which the patients could change their sorting principle once but retracted to the irrelevant one (see Table 1 for an example).

A total of 57.5% of the perseverative errors in this study were SIS-PE and 42.5% of the perseverative errors were Rec-PE. The correlation between the SIS-PE and Rec-PE was not significant (r = 0.25). Thus, these two perseverative scores seem to reflect different aspects of perseverative behaviour as previously mentioned.\(^7\) \(^7\) \(^8\) We finally therefore used the scores of the SIS-PE and Rec-PE for the subsequent image analyses.

**SPECT scanning**

Within a month of the neuropsychological evaluations, the resting rCBF in all the subjects was evaluated using single photon emission computed tomography (SPECT). Subjects lay on the scanner bed with their eyes closed and 740 MBq of \(^{99m}\text{Tc}\)hexamethyl-propyleneamine oxime (HMPAO) was administered intravenously. After 15 min of injection, the SPECT image was acquired over a period of 20 min using a triple-head SPECT scanner (GCA-9300AUI; Toshiba Medical, Tokyo, Japan) with high-resolution fan-beam collimators. The axial field of view of the cameras was 22 cm and the resolution of the system was 7.5 mm full width at half maximum (FWHM) in the centre of view. Projection data, collected in a 128\(\times\)128 matrix, were prefiltered with a Butterworth filter with order 8 and a cut off frequency of 0.11 cycles/pixel. Transaxial images were reconstructed with a Ramp back projection filter. The post-reconstruction attenuation correction was not applied. The reconstruction yielded 1.7\(\times\)1.7\(\times\)1.7 mm voxels with a 128\(\times\)128 matrix and 80 slices.

**Image processing**

The SPECT data were processed with Statistical Parametric Mapping (SPM2, Wellcome Department of Cognitive Neurology, London, UK). All the SPECT images were registered and transformed into a standard stereotactic anatomical space (International Consortium of Brain Mapping space)\(^4\) using the original disease-specific SPECT template (made from the SPECT images of 33 AD and nine MCI patients). The resulting voxel size was 2\(\times\)2\(\times\)2 mm. The images then were smoothed with a 10 mm gaussian filter to reduce the variance due to individual anatomical variability. Thereafter, the effect of differences in global CBF across subjects was removed using proportional scaling. This process equalised the mean CBF value to 50 ml/dl/min across subjects, and generated an associated adjusted error variance for each voxel; therefore this allowed us to make comparisons of the mean CBF distributions across all subjects.

Although the regional cerebral metabolism and rCBF could be affected by neuronal lesions—that is, cerebral atrophy—as well as synaptic dysfunction, all studies that performed voxel-based atrophy correction of glucose metabolism have concluded that the reduced cerebral metabolism represents a true loss of functional activity and not simply an artefact caused by brain atrophy.\(^3\) \(^6\) Moreover, some correlational studies (not voxel based) took brain atrophy into account in their analysis and the results were nearly the same as those obtained without correction for atrophy;\(^3\) \(^7\) \(^8\) we therefore did not perform atrophy correction in the subsequent analyses.

**Voxel by voxel parametric analysis**

The parametric approach models changes in rCBF as a function of the experimental parameter by using regression analysis. In general, SPM uses the general linear model to build F- or t-statistic fields—that is, SPM(F) or SPM(t).\(^4\) In the special case of parametric studies it is used to make statistical inferences about the correlation of rCBF and a study parameter. In the present study, we modelled changes in the rCBF with a non-linear function of the WCST performance parameters using a regression with a second-order polynomial function. As we expected that rCBF in the patient’s brain will decrease monotonically in relation to the increase of their WCST errors, voxel-specific t-statistics were used to make inferences about the negative non-linear regression of rCBF on the study parameters. These analyses generated statistical parametric maps of the t-statistic (SPM(t)), which were subsequently converted to unit normal distribution (SPM(Z)). Age and the MMSE scores were treated as covariates of no interest, so that the confounding effects of age and dementia severity on the rCBF distribution were covaried out. In this analysis, the statistical threshold was set at p<0.001 (Z>3.09) without correction for multiple comparisons. This threshold is regarded as sufficiently conservative to protect against false-positive results in PET analysis.\(^9\)

The stereotactic coordinates of Talairach and Tournoux\(^1\) were used to report the brain areas, but descriptions of the anatomical location were also based on visual inspection of the standard structural MRI.

**Volumes of interests correlation analysis**

To clarify which of the prefrontal areas was actually associated with the WCST performances, we evaluated correlations between the rCBF in several predetermined prefrontal regions and the first and second order polynomial expansion of the WCST error scores. MRicro software (Rorden C, http://www.mricro.com) was used for the volume of interests (VOI) analyses. Four different prefrontal areas that can be associated with cognitive processes in the WCST were determined based on the previous Neuroimaging studies: (1) rostrodorsal PFC (x = ¡34, y = 50, z = 14), which is an anterior part of the middle frontal gyrus near the frontal pole, and regarded as BA 10 or most rostral part of BA 46; (2) posterior PFC (x = ¡46, y = 8, z = 28), which was situated in the inferior frontal sulcus near the junction with the precentral sulcus and corresponded to BA 8A/44 or 45; (3) ventrolateral PFC (x = ¡36, y = 28, z = ¡2), which is located around the oppercular part of the inferior frontal gyrus, and regarded as Brodmann’s area (BA) 47/12; and (4) anterior cingulate area (x = ¡8, y = 20, z = 46). The activation peaks in the previous fMRI and PET studies related to the WCST were selected\(^1\) \(^4\) \(^1\) \(^4\) \(^4\) \(^4\) \(^4\) and averaged to yield the representative stereotactic coordinates for each prefrontal region. Then, the coordinates were slightly modified to be positioned on the cortical CBF of the SPECT images in the present study.

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**Table 1: Examples of responses scored if colour was the correct sorting principle and figure the previous one**

<table>
<thead>
<tr>
<th>Responses</th>
<th>Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>C, colour</td>
<td>Correct</td>
</tr>
<tr>
<td>F, figure</td>
<td>SIS-PE</td>
</tr>
<tr>
<td>N, number</td>
<td>Non-perseverative error</td>
</tr>
<tr>
<td>F</td>
<td>Rec-PE</td>
</tr>
<tr>
<td>N</td>
<td>SIS-PE</td>
</tr>
<tr>
<td>N</td>
<td>SIS-PE</td>
</tr>
</tbody>
</table>

C, colour; F, figure; N, number; Rec-PE, recurrent perseverative error; SIS-PE, stuck-in-set perseverative error.
Spherical VOIs (20 mm in diameter) centred on the predetermined prefrontal regions were drawn on all 10 mm smoothed, anatomically and globally normalised SPECT images, and every voxel value in the VOI was averaged to calculate the rCBF. To determine the relation between the WCST parameters and the rCBF, we evaluated partial correlation coefficients between the WCST performances and the rCBF, including age and the MMSE scores as confounding parameters. For the partial correlation coefficients, statistical significance level was set at $p<0.01$.

RESULTS
The SPM analyses demonstrated that the SiS-PE scores showed significant non-linear regression with the rCBF in the right rostrodorsal PFC (rostral part of the middle frontal gyrus, the coordinates, $Z$ score, and the cluster size ($k$) were $x = 32$, $y = 48$, $z = 10$, $Z = 3.36$, $k = 118$ voxels), left rostrodorsal PFC (the middle frontal gyrus, $x = -32$, $y = 58$, $z = 20$, $Z = 3.29$, $k = 11$ voxels), and the left frontopolar cortex (the frontomarginal gyrus, $x = -26$, $y = 52$, $z = -6$, $Z = 3.43$, $k = 39$ voxels)(fig 1). In contrast, the Rec-PE showed significant regression with the rCBF in the left inferior parietal cortex (around the angular gyrus, $x = -38$, $y = -56$, $z = 40$, $Z = 3.27$, $k = 35$ voxels)(fig 2). Even when the statistical threshold was lowered to $p<0.01$, the Rec-PE was not associated with the rCBF in the prescribed frontal cortices, whereas the SiS-PE was only related to the reduced rCBF in these prefrontal regions. These results were unchanged when the three FTD patients were excluded from the analyses.

The VOI analyses also demonstrated that the rCBF in the right rostrodorsal PFC showed significant non-linear correlation with the SiS-PE score ($r = -0.355$, $p = 0.0025$). The non-linear correlation between the rCBF in the right rostrodorsal PFC and the SIS-PE score approached to the significance level ($r = -0.293$, $p = 0.013$). The linear correlation between the rCBF in the right rostrodorsal PFC and the SIS-PE score also approached to the significance level ($r = -0.30$, $p = 0.011$). The rCBF in the other prefrontal areas did not show any significant correlations with the SIS-PE score. The Rec-PE did not show any significant correlations with the rCBF in the prefrontal areas in the VOI analyses.

DISCUSSIONS
We determined the sites of significant correlations between two different types of the WCST perseverative errors and rCBF in the group of degenerative dementia. First, the number of SIS-PE score was significantly associated with the reduced rCBF in the rostrodorsal PFC, supporting the conclusion in our previous fMRI study in healthy subjects.14 Second, the Rec-PE was correlated to the left parietal activity but not to the prefrontal activity, suggesting its differential psychophysiological nature from the SIS-PE.

We pooled SPECT data from four different clinical entities of AD, MCI, FTD, and healthy elderly for the present analyses. This involvement of the heterogeneous groups enabled us to extract the brain regions that are associated with the WCST impairments, independently of clinical diagnosis. The essence of this approach is that the basic units of classification in psychopathology are neither diseases nor syndromes but psychological dysfunctions, and that shared behavioural deficits in nosologically distinct neuropsychiatric disorders may have common neurophysiological correlates.42 Within this framework, the previous study showed that poverty of speech is associated with decreased rCBF in the left dorsolateral PFC in depression and schizophrenia in a manner that is independent of diagnosis.43
The correlation of rCBF decrease with the impaired WCST performances in the present study could be confounded by disease severity and ageing effect. Many neuroimaging studies have shown that the distribution of the rCBF decrease depends on the stage of dementia. In moderate to severely demented AD patients, frontal hypometabolism is as severe as that in the parietotemporal area.44 45 Ageing is also associated with the rCBF decline in the frontotemporal cortex.46 47 Therefore, one could argue that the rCBF decrease in the several brain regions, such as the rostrodorsal PFC, may be because of progression in dementia itself and does not truly relate to neuropsychological performances. However, this is unlikely because of the following reasons. First, in AD (a large number of patients in this study) the most consistent finding across the neuroimaging research was that the gradual rCBF decline according to the disease progression was observed in the parietotemporal association cortex.44 45 If the rCBF decrease is associated with progression of dementia, it should be detected in the posterior association cortices. However, the reduction of the rCBF in relation to the SIS-PE was observed only in the prefrontal cortex. Second, the WCST performances in our sample showed no significant correlation with dementia severity—that is, MMSE score—or age (data not shown). In addition, the influence of age and dementia severity was controlled by setting age and the MMSE score as confounding variables in all the regression analyses.

Differential involvement of the prefrontal regions

A principal aim of this study was to investigate the neural correlates of two distinct kinds of perseverative errors implicit in the WCST. The results of the present study unequivocally demonstrate that the SIS-PE score is particularly associated with the rCBF reduction in the bilateral rostrodorsal PFC (right > left) and the left frontopolar cortex. As the SIS-PE reflects failure of shifting attentional set away from a previously reinforced stimulus dimension towards a previously irrelevant stimulus dimension, these findings augment evidence that attentional set shifting is crucially mediated by predominantly the rostrodorsal PFC. Stuss et al7 reported that the right frontal patients had significantly elevated “perseveration to the preceding response” score in the WCST, which was defined similar to the SIS-PE in this study, whereas very few non-frontal patients exhibited these errors. Our previous study in normal subjects revealed that the right rostrodorsal PFC was activated only at the time of attentional set shifting and that it may be a critical area in shifting an attentional set between different perceptual dimensions.14 The present results complement and strongly support our previous findings, and suggest that the SIS-PE would be a true pathognomonic sign of frontal dysfunction.

Previous findings in the other imaging studies are also consistent with this notion. PET studies in normal subjects have shown that the rostrodorsal PFC was consistently activated during the performance of the WCST.10 11 41 In the event-related fMRI experiment, Konishi et al13 showed that transient neural activity was observed in the left frontal cortex, including the rostrodorsal PFC at the time of updating cognitive set during the WCST. Further support for a contribution of this brain region on the perceptual...
dimensional change comes from a recent study that compared cross-dimensional changes with within-dimensional changes in a sophisticated set shifting task analogous to the WCST. The comparison of extradimensional and intradimensional shifts revealed a right dorsolateral prefrontal activation (BA 9/46), which was very close to our rostrodorsal PFC activation, suggesting that control of attentional set is crucially mediated by this prefrontal region.

In addition to the rostrodorsal PFC, the rCBF reduction in the left frontopolar cortex was also associated with the increased SiS-PE. The possible involvement of the left frontopolar cortex during the cross-dimensional shifts is supported by several previous studies. Previous PET studies demonstrated that the left frontopolar activation was observed during the performance of WCST. A recent fMRI study of visual singleton (odd-one-out) feature search found that the left frontopolar cortex appears to be exclusively involved in the process of attentional switching from one visual dimension to another.

Previous fMRI studies of the WCST showed that the neural activity in the posterior PFC and the ventrolateral PFC also increased during set shifting trials. However, both SPM and VOI analyses in the present study failed to show any significant correlation between the rCBF in these regions and the perseverative error scores in the WCST, suggesting these prefrontal subregions other than the rostrodorsal PFC may play different roles in the attentional set shifting. Cumulative evidence suggests that the posterior PFC is less specific to the attentional switching and may be involved in the selection of the appropriate response based on the currently relevant rule. The ventrolateral PFC region is commonly activated if it is necessary to plan a response to the negative feedback during the type of go/nogo tasks or reversal learning task. In the monkey, lesions of the ventrolateral prefrontal convexity impaired the spatial and non-spatial reversal learning. Furthermore, a recent study by Cools et al showed that the ventrolateral PFC is primarily associated with reversal learning and is not explained by negative feedback. Thus, the ventrolateral PFC may be involved in shifting of lower level stimulus reward associations—that is, reversal learning—whereas higher level cognitive set shifting is mediated by the rostrodorsal PFC, which was the only prefrontal subregion showing a significant correlation with the SiS-PE scores in the WCST. This difference in the dorsolateral versus ventrolateral PFC involvement in the WCST is consistent with a theory that emphasises the difference in the role of the mid-dorsolateral prefrontal area, which is necessary for the monitoring of information in working memory, as opposed to the midventrolateral prefrontal area, which is involved in more basic executive processes, such as the active comparison of stimuli held in working memory.

**Different types of perseverative errors**

Another important finding in the present study was that the Rec-PE was differentially associated with the rCBF reduction in the left inferior parietal cortex and showed no significant correlation with the rCBF in the prefrontal region. Although we cannot exclude that this correlation is false positive, involvement of the parietal cortex was reported in previous PET and fMRI studies of the WCST. Sandson and Albert suggested, based on their study of brain damaged patients, that the recurrent perseveration is linked to left temporal-parietal damage and not to frontal damage. Several studies in healthy subjects showed that the parietal cortex is involved in processing of visual attention. Fink et al revealed that object based and space based attention share common neural mechanisms in the left lateral inferior and bilateral medial superior parietal areas. Wojciulik et al examined brain activation during two different spatial attention tasks and a non-spatial attention (conjunction and a feature) task to determine whether different kinds of visual attention rely on a common neural substrate. All spatial and non-spatial attention tasks activated the inferior parietal lobules (the junction of intraparietal and transverse occipital sulci and the anterior intraparietal sulcus) bilaterally. They emphasised that these parietal regions play a more general role in visual attention, and suggested that the parietal cortex may perform an inhibitory function in selective attention suppressing task irrelevant distractors. This theoretical position may be consistent with the results in the present study because the Rec-PE in the WCST may be closely related to failure to suppress the previously relevant but currently irrelevant visual dimension. Although the relation between the type of perseverative errors on the WCST and the localised parietal lesion has seldom been examined, impairment on the WCST was reported in one patient with left parietal damage (patient TW1124). The present results suggest that the SiS-PE and Rec-PE may be differentiated not only behaviourally but also on their neurophysiological basis. These two types of perseveration should be separately estimated in future studies of the WCST in brain lesioned patients.

**Right versus left prefrontal cortex in the WCST**

The results of the present study and our previous fMRI study suggest right frontal predominance in the process of the attentional set shifting. The right dominant tendency in the WCST has emerged in several studies, although some studies found the opposite hemispheric effect. Stuss et al recently demonstrated that patients with either left or right focal prefrontal lesion were impaired on the “perseveration to the preceding response” score in the WCST, but the right prefrontal group was impaired more severely than the left prefrontal group. It is possible that the discrepancy of the hemispheric effect among previous studies is associated with verbal mediation during the performance of the WCST. Several neuroimaging studies suggested that when abstract—that is, difficult to verbalise—figures or colours are used in the tasks and covert verbalisation is effectively prohibited, the right dorsolateral PFC is differentially activated in relation to the attentional set shifting. In contrast, the bilateral PFC is active during the performance of the original WCST. These findings suggest that although the essential process of the visual dimensional changes is mediated by the non-verbalsystems in the right PFC, the set shifting in the WCST could be also performed through the verbal systems in the left hemisphere.

Overall, the present data provide, along with the previous results of activation study in healthy subjects, converging evidence concerning the close relationship between the failure in shifting attentional set and the dysfunction in the rostrodorsal PFC. Furthermore, this study showed that the Rec-PE may not be associated closely with the function of the prefrontal cortex, suggesting that the SiS-PE and Rec-PE should be differentially estimated in the WCST. From these neuropsychological standpoints, future re-examination of the WCST performances in the focal brain damaged patients would be of considerable interest.

**Authors’ affiliations**

Y Nagahama, T Okina, N Suzuki, M Matsuda, Department of Geriatric Neurology, Shiga Medical Center, 5-4-30 Miyamori, Moriyama-city, Shiga 524-8524, Japan

H Nabatame, Department of Neurology, Shiga Medical Center

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