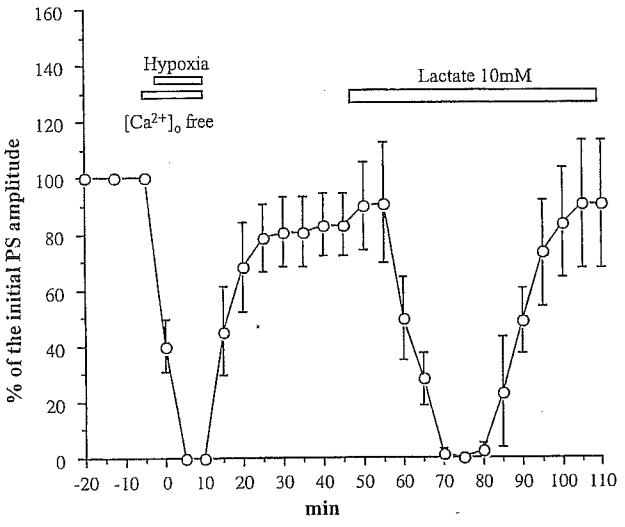

Fig. 3. Effect of the omission of extracellular calcium during hypoxia on the amplitude of population spikes during subsequent replacement of glucose with lactate. Calcium was omitted from the perfusion medium containing 5 mM glucose and 200 μM EGTA was added (to remove any free Ca^{2+}) 5 min before start of hypoxia as indicated by open horizontal bars. Zero minute on the horizontal axis indicates the start time of hypoxia. After recovery of PS amplitude, the perfusion medium was replaced with glucose-free medium containing 10 mM lactate as shown. The ordinate is the same as in Fig. 1. Each plot indicates the mean value ±S.E.M. of five slices.

and VSCCs. We did not observe the same phenomenon after ischemia-like conditions (hypoxia and glucose-free medium); recovery from such a severe challenge is rare (data not shown).

To further elucidate the role of Ca^{2+} influx through the NMDA receptor and VSCCs, slices were subjected to hypoxia with 5 mM glucose, but no Ca^{2+} in the perfusion medium (Fig. 3). The time course of the PS amplitude exhibits a transient block of PS with recovery during lactate replacement for glucose, indicating that the effect of the low glucose level seen in Fig. 1 is negated without extracellular calcium. The level of 5 mM glucose itself is sufficient for maintenance of PS in the dentate gyrus of hippocampus (Kanatani et al., 1995; Li et al., 2000). These results demonstrate that the induction of lactate-supported PS is dependent on Ca^{2+} influx through the NMDA receptor and the VSCC, and that the small reduction of glucose levels (from 10 to 5 mM), which does not influence the basic PS amplitude, triggers the activation of these channels during hypoxia.

3.2. Hypothermia can prevent the induction of lactate utilization for PS

Mild hypothermia (33–29 °C) drastically reduces Ca^{2+} accumulation in the cell and improves the recovery of PS after oxygen and/or glucose deprivation (Takata et al., 1997). Furthermore, we previously reported that hypothermia prevented the decline of PS and ATP levels in the slices, particularly during glucose deprivation. These results suggest that a component of the mechanism used to sustain PS and ATP levels by anaerobic glycolysis is highly temperature sensitive (Takata et al., 1997). Based on these observations, we proposed that hypothermia will prevent the reduction of ATP levels that may trigger the activation of the NMDA receptor and the VSCC. First, we tested the effect of hypothermia on PS amplitude in lactate medium at 30 °C. Upon lowering the temperature to 30 °C with 10 mM glucose, an initial mild transient depression (80% of the initial amplitude) of PS is observed, after which the amplitude rises to 120% (Fig. 4A). The temperature of 30 °C was chosen because a previous report by Aihara et al. (2001) showed that, within the range of 17–36 °C, 30 °C resulted in PS with the highest amplitude. Indeed, the degree of increase of PS in our experiments is almost identical with their results. By selecting the temperature at which the amplitude of PS is maximized, we can see easily the response in PS when there is a depressive effect from energy deprivation. Next, lactate was introduced and the amplitude of PS initially increased to 140% and subsequently stabilized at 120–130% (Fig. 4A). This result confirms that lactate can maintain synaptic potentials at 30 °C for at least 60 min as in the case of glucose deprivation with hypothermia. When hypothermia is introduced during hypoxia with 5 mM glucose in the perfusion medium, the PS amplitude is reduced to 60% and recovers fully after recirculation of standard oxygenated medium at 35 °C. After replacement of glucose with lactate, a transient blockade and subsequent spontaneous recovery of PS results (Fig. 4B). This suggests that hypothermia prevents the exhaustion of energy originating from anaerobic glycolysis and maintains the essential energy levels that prohibit the induction of lactate-supported synaptic potentials.

3.3. ATP levels in the DG region

Lowering the glucose levels in the medium during hypoxia enables PS to be maintained at the original levels in the lactate medium, however, hypothermia prevents this maintenance effect. We next determined the energy (i.e., ATP) levels in the slices under both conditions. The DG region of each slice was selectively dissected and incubated for 10 min in standard medium (containing 10 mM glucose), glucose-free medium (ischemia-like conditions), or 5 mM glucose medium bubbled with 95% N_2/5% CO_2 (hypoxic conditions). The ATP concentration of each sample was determined by a sensitive microassay method (Okada, 1974). ATP levels in the slices are 13.6 ± 0.55 mmol/kg protein under the control conditions (10 mM glucose bubbled with 95% O_2/5% CO_2). ATP levels were reduced in each experimental case relative to the control. The ATP level was 12.8% of the control level under ischemia-like conditions, 36% of the control in 5 mM glucose medium with hypoxia, and 66.6% of the control in 10 mM glucose medium with hypoxia (Fig. 5). When hypothermia is introduced with the 5 mM glucose-containing medium, the

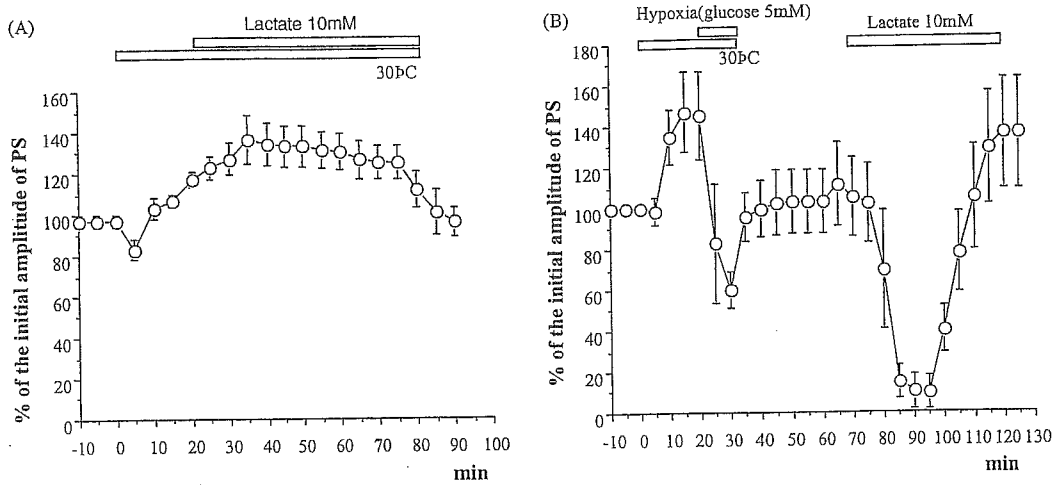

Fig. 4. (A) Effect of hypothermia on the amplitude of population spikes over time. Hypothermia at 30 °C was introduced and 10 mM glucose in the perfusion medium was replaced with lactate as indicated by the open horizontal bars. (B) Effect of hypoxia with 5 mM glucose combined with hypothermia on the amplitude of population spikes after replacement of glucose with lactate. Hypothermia at 30 °C was introduced prior to hypoxia with 5 mM glucose. After recovery from hypoxia, glucose was replaced with lactate as indicated by the open horizontal bars. The ordinate is the same as in Fig. 1. The last half of control data (10 min out of 20 min observation) is shown in both (A) and (B). Each plot indicates the mean value ± S.E.M. of five slices.

ATP levels are significantly preserved at 64.9% of the control levels ($P < 0.001$; Fig. 5). These results indicate that hypothermia preserves the energy levels in the slices during hypoxia and that the energy levels measured in the slices during hypoxia correlate with the subsequent induction of lactate-supported synaptic potentials.

3.4. Glutamate release from the DG region

Energy deprivation, such as hypoxia or hypoglycemia, induces extracellular glutamate accumulation (Choi, 1988; Katayama et al., 1991; Takata et al., 1995). We previously demonstrated that extracellular glutamate levels increased when lactate was substituted for glucose (Takata et al., 2001). To investigate the correlation between extracellular glutamate levels and the induction of lactate utilization after hypoxia with low glucose in the medium, the glutamate levels in the medium under different conditions were determined using HPLC. The basal level of glutamate released in the medium is 1.17 ± 0.075 μM/mg protein. After 10 min of incubation under ischemia-like conditions, the released levels of glutamate modestly increase and prominently increase after 30 min. The glutamate levels in the medium with 5 mM glucose do not increase even after 30 min exposure to hypoxia (data not shown).

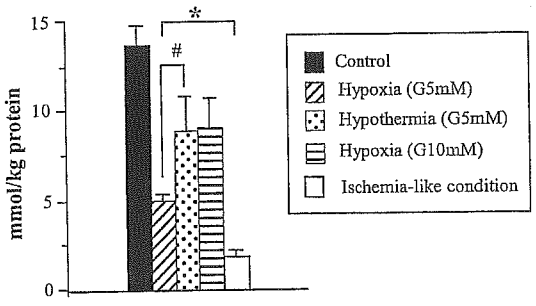

Fig. 5. Concentration of ATP from dentate gyrus of hippocampus during hypoxia with 5 mM glucose (G5mM), 10 mM glucose (G10mM), G5 mM + hypothermia, and ischemia-like conditions (hypoxia + glucose-free). The ordinate is the average ATP concentration (in mmol ATP/kg protein) ± S.E.M. from four dissected parts of dentate gyri from hippocampus. *Significantly different when compared with the control. #Significant difference between the 5 mM glucose and 5 mM glucose + hypothermia groups.

4. Discussion

Saitoh et al. (1994) found that the PS amplitude measured from the granule cell of dentate gyrus from the guinea pig hippocampus spontaneously recovered after a transient blockade when glucose in the perfusion medium was substituted with lactate. Kanatani et al. (1995) confirmed that glucose metabolites such as fructose, pyruvate and lactate preserved ATP and creatine phosphate levels in the slices although the PS amplitude was not maintained. We investigated further using intracellular recording techniques and reported that the spontaneous recovery of synaptic potentials following lactate substitution for glucose was not observed in CA3 pyramidal neurons and that lactate could not support synaptic potentials (Takata and Okada, 1995). Early studies proposed that the decline of ATP levels is responsible for the suppression of the synaptic potentials during energy deprivation (Lipton and Whittingham, 1982; Martin et al., 1994). However, the fact that synaptic potentials cannot be supported despite the maintenance of energy levels suggests that reduced ATP is not the only explanation for the loss of synaptic potentials. We proposed that there are distinct roles for anaerobic and aerobic glycolysis and reported that there is a difference in the mechanism by which synaptic potentials are suppressed when the cells are deprived of glucose versus oxygen that

cannot be explained solely by energy metabolism (Takata and Okada, 1995). Furthermore, a switching mechanism between anaerobic and aerobic glycolysis that is dependent on the NMDA receptor and VSCCs (Takata et al., 2001) is suggested since lactate can support synaptic potentials after exposure to glucose deprivation (Sakurai et al., 2000; Takata et al., 2001). Yamane et al. (2000) demonstrated that damaged hippocampal slices containing low levels of ATP could use lactate for maintenance of PS. Summarizing these observations, the induction of lactate utilization for maintenance of synaptic potentials is triggered by conditions of limited glucose such that the energy source dependency may change from glucose alone (adult form) to utilization of glucose and/or lactate that may be called, immature form.

4.1. Effect of lowering glucose levels during hypoxia on induction of lactate-supported PS

When lowering glucose levels to 5 mM in the perfusion medium, exposure of the hippocampal slice to hypoxia induces the utilization of lactate for the maintenance of PS. This is in contrast with the result from the medium containing 10 mM glucose, in which lactate cannot support the PS amplitude (Fig. 1). The effect of lower glucose levels is abolished by applying antagonists of the NMDA receptor and the VSCC (Fig. 2) in the perfusion medium, omission of Ca^{2+} from the perfusion medium (Fig. 3), or introduction of hypothermia during hypoxia (Fig. 4B). Because the antagonists of the NMDA receptor and the VSCC block the induction of lactate-supported potentials following hypoxia, we propose that the activation of these channels is necessary for the induction of lactate usage for maintenance of PS as in the case of glucose deprivation (Takata et al., 2001). Furthermore, the failure of lactate to maintain PS after hypoxia exposure in calcium-free medium supports the involvement of these channels.

4.2. Hypothermia also blocks the induction of lactate usage for PS maintenance

We further examined the effect of hypothermia on the induction of lactate utilization to support potentials. Hypothermia reduces ischemic brain damage (Busto et al., 1987), and a small reduction in the temperature significantly improves the recovery of field potentials and retains the energy levels of hippocampal slices during oxygen and/or glucose deprivation (Takata et al., 1997). Furthermore, reducing the temperature from 35 to 30 °C reduces the rate of energy usage in these slices by about 30% (Okada, 1988). To simulate the conditions of energy conservation during hypoxia, the temperature of the incubator containing 5 mM glucose medium was reduced to 30 °C (Fig. 4B). Hypoxia increases the basal level of the PS amplitude (Aihara et al., 2001), reduces the PS decline during hypoxia and prevents the induction of lactate usage for PS maintenance (Fig. 4B). This indicates that the mechanism used to preserve energy prevents the induction of lactate usage for PS maintenance.

4.3. ATP levels in the dentate gyrus during hypoxia with low glucose

Hypoxia induces an accelerated rate of glycolysis to compensate for the lack of energy production from the TCA cycle (a Pasteur effect). Although it also results the decline of ATP levels of hippocampal slices (Fig. 5), the PS amplitude shows full recovery after reintroduction of the standard medium. Under ischemia-like conditions when the ATP level falls to approximately 2 mmol/kg protein (Fig. 5), the PS cannot be evoked again following recirculation of normal oxygenated medium after up to 60 min (data not shown). There may be a critical ATP level in the range of 2–5 mmol/kg protein in our experimental setting after which the PS does not recover. Wang et al. (2000) reported that an ATP level of approximately 1 nmol/mg dry weight protein (note: wet weight protein is used in our report) determines the reversibility of synaptic potentials in the CA1 pyramidal neurons. In comparing the original ATP levels reported by Wang et al. (2000) (6.1 nmol/mg protein) to our original ATP levels (13.6 mmol/kg protein) the levels that determine synaptic potential irreversibility may be similar (16% versus 15% of the original levels). We have found that the ATP level is significant not only for determining whether synaptic function can be recovered, but it is also correlated with the energy source dependency (adult or immature form) at higher levels (5 mmol ATP/kg protein or 37% of the initial levels) under these experimental conditions. The ATP levels are identical in experiments using 5 mM glucose + hypothermia to those using medium containing 10 mM glucose under hypoxia (Fig. 5). This also explains the inverse correlation of ATP levels and the induction of lactate-supported PS.

4.4. Extracellular glutamate concentration during hypoxia

We reported previously that a blockade of synaptic potential during hypoxia alone was not accompanied with a robust increase of intracellular calcium ($[Ca^{2+}]_i$), as in the case of glucose deprivation or combined oxygen + glucose deprivation (Takata and Okada, 1995). Compared with combined oxygen + glucose deprivation, a hippocampal slice exposed to hypoxia alone has the potential to recover synaptic potentials in the presence of a sufficient supply of glucose (Tian and Baker, 2000). The moderate decrease in ATP levels compared with that seen under ischemia-like conditions (Fig. 5) prevents the reverse action of the glutamate transporter or blocks the glial glutamate transporter (Erecinska and Dagani, 1990). It is surprising that NMDA and VSCC antagonists prevent the induction of lactate-supported PS in the apparent absence of extracellular glutamate release during hypoxia under the conditions of

lower glucose. However our measurement of the glutamate in the medium may not precisely reflect the concentration of extracellular glutamate in the slice. Using glutamate oxidase attached to a micro-probe, we reported the increase of extracellular glutamate from the CA3 dendritic region 10 min after the initiation of glucose deprivation (Takata et al., 1995) when the ATP levels in the slice are well preserved (Takata and Okada, 1995). While these results indicate that mild extracellular glutamate release triggering the induction of lactate-supported PS may occur during hypoxia with lower glucose levels, more detailed investigations will be required to substantiate this observation.

In summary, our present experiments confirm our previous conclusions that lactate cannot be utilized initially as a primary substrate to maintain synaptic activity instead of glucose in the adult hippocampal neurons, especially the CA3 pyramidal neurons and the granular neurons of dentate gyrus. It still remained to be proved that whether the CA1 pyramidal neurons show the similar characteristic with the paradigm we used here in current experiment. However, the significant point overlooked so far by other investigators is that lactate can maintain energy levels in hippocampal slices, but fails to be a complete alternative to glucose for the maintenance of synaptic function in immediate prepared slices (see Section 1, Yamane et al., 2000) and at physiological temperature (35–37 °C). Moreover, conditions of glucose shortage, such as hypoxia with reduced glucose or glucose deprivation, triggers a shift in substrate utilization from the adult form (glucose alone) to the immature form (lactate as well as glucose, Wada et al., 1997). We also previously demonstrated that pyruvate and ß-hydroxybutyrate as well as lactate could not sustain the synaptic potential despite the fact that energy levels of the slices were well-maintained in the adult neuron (Kanatani et al., 1995; Wada et al., 1997). However, immature neurons can maintain both synaptic activity and energy levels using these substrates. Studying this dynamic shift in energy metabolism from the adult to the immature form in adult neurons will have a great impact on the understanding of the pathophysiology of neurons injured under post-ischemic or degenerative conditions.

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Tohoku J. Exp. Med., 2005, **205**, 205-214

Invited Review

Medical Treatments and Cares for Geriatric Syndrome: New Strategies Learned from Frail Elderly

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KUBO, H., NAKAYAMA, K., EBIHARA, S. and SASAKI, H. *Medical Treatments and Cares for Geriatric Syndrome: New Strategies Learned from Frail Elderly.* Tohoku J. Exp. Med., 2005, **205** (3), 205-214 —— In Japan, there are 21 million older people above 65 years, and about 8% of them are frail elderly. Geriatrics is to study the frail elderly as to why they become frail elderly, and to treat patients properly or the remaining 92% older people not to become frail elderly. In order to promote health of the older people, geriatricians have to take deep insights for cares as well as medical treatments. With such a will, we find the way to prevent diseases in the older people. In this review, we describe medical treatments and cares for promoting successful aging. ———— geriatrics; dysphagia; pneumonia; depression; Alzheimer's disease; frail elderly

While a single organ disease may cause various symptoms in young patients, in older patients complicated associations of multiple organ diseases generally cause a single symptom. The frequent problems in the frail elderly are eating problems, aspiration, pneumonia and associated brain disorders. Those geriatric syndromes could be explained with brain disorders. The elderly patients are most ill and most complex, and it is hard to treat the elderly properly. Nevertheless, recent studies make it possible to promote health of the older people.

Dysphagia

Eating generally takes longer in the frail elderly. We find that their reduced appetite may be caused by glucose-intolerance due to age-related impairment of the pre-cingulate gyrus (Hu et al. 2002). Furthermore, some of these patients are unable to swallow food properly. Following insertion of food into their mouths, they only chew it for a short while (He et al. 2004). These subjects might have dysfunction of the pre-insula region (Okamura et al. 2004). The question remains as to how we can treat them to recover their appetite? Aromatherapy with black pepper stimulates the pre-cingulate gyrus and pre-insula regions and improves glucose metabolism that results in the recovery of appetite. This is a method that is effective in some of the elderly to promote meal eating. Acupuncture to stimulate planters, lateral to the knees or medial to the calces is one strategy to promote swallowing, so that the residue in the mouth reduces and aspiration is prevented (Seki et al. 2003). Although it improves their appetite and swallowing function somewhat, these activi-

Received December 9, 2004; revision accepted for publication December 28, 2004.
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ties remain problematic for some.

Aspiration occurs when some of food passes into trachea, instead of the esophagus. This does not improve by eating more food, since it is caused by impaired pharyngeal reflexes (swallowing reflex) for adequate swallowing (Nakazawa et al. 1993), and also impaired cough reflex in order to expel aspirated foreign bodies (Sekizawa et al. 1990). To contend with this phenomenon in pulmonary medicine, the Cough Referral Guideline was developed in the US. This guideline discusses the management of persistent or recurrent cough. However, the elderly often have difficulty coughing even when treated with a citrate aerosol that strongly induces coughing. However, the cough guidelines in the US do not relate at all to difficulty of coughing. In fact, difficulty of coughing is more likely to cause aspiration pneumonia that can result in death. The reason why they do not mention it is they do not take the elderly into consideration. The importance of this phenomenon must be considered, when caring for the elderly.

Aspiration

One cause of impairment of swallowing (Jin et al. 1994) and the cough reflex (Ujiie et al. 1993) that can cause aspiration is decreased production of substance P in the sensory nuclei of the vagus and glossopharyngeal nerves in the cervical ganglion (Sekizawa et al. 1997). Reduced substance P is caused by decreased production of dopamine in the cerebral basal ganglia that results from cerebrovascular disease in the cerebral deep cortex (Kobayashi et al. 1994; Jia et al. 1998). Therefore, one could say that aspiration caused by brain dysfunction (Yamaya et al. 2001a). Since reduced substance P leads to dysfunction of swallowing and the cough reflex, administration of substance P should resolve this problem (Nakagawa et al. 1995). One exogenous stimulant of substance P release is capsaicin, the key active component of red pepper (Ebihara et al. 1993). Oral administration of capsaicin permits these to be awake and improves swallowing and the cough reflex. Based on these data, we created a capsaicin lozenge with a hole in the center, so as to prevent suffocation in case of aspiration. When the lozenge was administered before meal, swallowing and cough reflex were significantly improved. A combination of black pepper, to stimulate appetite, and the lozenge, may be even more effective.

When assisting the elderly to eat, allowing hot foods to cool down before they eat is not appropriate, because of the impaired swallow reflex. Hot or cool temperatures can accelerate generation of substance P and evokes the swallow and cough reflex (Watando et al. 2004a). This is one strategy to take care of the elderly.

Pneumonia

Patients with pneumonia often exhibit weakness of swallowing or decreased frequency of swallowing (Watando et al. 2004a). Therefore, administration of substance P should improve their dysphagia. Tanatril®, an angiotensin-converting enzyme (ACE) inhibitor, has been shown to suppress degradation of substance P and elicits cough as side effect (Ebihara et al. 1996). We administered Tanatril® to people with impaired swallowing and cough reflex (Nakayama et al. 1998). The results showed that swallow function doubled to within the normal range. We compared between groups treated with and without Tanatril® and found that the group treated with Tanatril® showed improvement of swallowing and the cough reflex, and reduced pneumonia by one-third (Sekizawa et al. 1998). Additionally, since dopamine levels in these people are lower (Kobayashi et al. 1996), we used Symmetrel® to promote production of dopamine (Sekizawa et al. 1999). We compared between a group treated with Symmetrel® for three years and untreated controls. The results indicated that the group treated with Symmetrel® experienced decreased incidence of pneumonia by one-fifth (Nakagawa et al. 1999).

Although antibiotics are used clinically for pneumonia in the elderly, Tanatril® or Symmetrel® are also options as combination therapy with antibiotics or alone as monotherapy. The result of our work suggested that administration of Tanatril® or Symmetrel® reduced the use of antibiotics by half

(Kanda et al. 2004). Furthermore, the incidence of MRSA and death by pneumonia was also reduced. Consequently, the use of Tanatril® or Symmetrel® would likely also increase hospital profits. Although the elderly who have pneumonia are generally treated with antibiotics, if occult aspirations continue, they often lead to deterioration of pneumonia (Kikuchi et al. 1994). Therefore, pneumonia in the elderly has become known as intractable. Care for the prevention of occult aspiration (Nakagawa et al. 1997), as well as treatment with antibiotics reduced medical expenses by two-thirds.

Cerebral infarction

The reports regarding the effects of cholesterol are controversial (White et al. 2000). However, one such report found that elevation of cholesterol improved patients' resistance against infection, and resulted in improving their survival. One additional problem is the progression of arteriosclerosis by homocysteine (Matsui et al. 2001). Homocysteine is known in the pediatric genetic disease, homocysteinuria. In the elderly, elevated homocysteine levels are detected occasionally, and this is a concern as a risk factor for arteriosclerosis (Yamaya et al. 2001b). Vitamin B12 and folic acid are effective at reducing homocysteine levels. Vitamin B12 is found in fish, and folic acid is abundant in vegetables. Ingestion of these prophylactic diets leads to enhanced metabolism, which reduces the level of homocysteine resulting in lower incidence of arteriosclerosis (Sato et al. 2001). Nevertheless some of the elderly do not get sufficient vitamin B12 and folic acid from their diets. We compared between residents in the Onagawa-cho, Miyagi prefecture, i.e., a fishing village, and residents in the mountains area of the Kyusyu prefecture. The people in the fishing village in the Miyagi prefecture eat five times more fish than the people in the mountains. Moreover, the people in a fishing village ingest significantly more folic acid from seaweeds, including *konbu*, which decreases their homocysteine levels, thus preventing cerebral infarction. Therefore, fish and vegetables are indispensable in diets for the elderly.

Insomnia

Pneumonia develops in the night, because the swallowing reflex decreases in the elderly with cerebrovascular disease in the deep cerebral cortex (Wang et al. 1998), whereas healthy people do not change in their swallowing reflex, even during deep sleep (Pinto et al. 1994). Medical examinations of the brain indicated that one-half of those patients who are more than 65 years old have cerebrovascular diseases, and that they have decreased swallowing reflex, even when they are awake in the daytime (Nakagawa et al. 2000). Regarding the cough reflex as a protective function, patients frequently complain that persistent coughs disturb their sleep. However, this is not true; if people are truly sleeping deeply, they never cough (Zheng et al. 1997). This raises the following question: Does less sleep prevent aspiration pneumonia? We investigated how long elderly people sleep (Manabe et al. 2000). This may sound strange to some people, but to elucidate the universal tendencies of the elderly in their daily life is our guiding principle. Despite their frequent complaints of insomnia, these results showed that they sleep a tremendously long time, 6 hours in the night and 3.5 hours in the daytime, totaling 9.5 hours in a day. We generally prescribe sleeping pills for the elderly patients that constantly complain of insomnia. Although it is safer while they are taking weak hypnotics, if these patients have to switch to stronger ones, due to tolerance induced by chronic treatment, strong hypnotics have been demonstrated to suppress dopamine resulting in a decline of the swallowing reflex (Wada et al. 2001). Although these patients appreciate the effectiveness of the medicine to help their sleep, they are occasionally readmitted to the hospital because of aspiration pneumonia resulting in much more serious conditions. We found that the elderly taking hypnotics exhibit three times greater incidence of aspiration pneumonia.

Oral care

The causes of pneumonia are endogenic for the elderly and exogenic for youths. Since endogenic pneumonias are caused by aspiration of

bacteria from their mouth, cleaning their mouth must be one of the prophylaxes. We recommended bushing of the teeth for five minutes after each meal, which resulted in improvement of their swallowing (Yoshino et al. 2001) and cough reflexes (Watando et al. 2004b). Oral function is associated with 40% of the sensory and motor areas of the brain. This study demonstrated for the first time, that stimulation of their oral cavity led to stimulation of their brain, and consequently enhanced their general function.

We compared between groups with and without oral care and found that the group having oral care had a 40% lower incidence of aspiration pneumonia (Yoneyama et al. 1999). These patients were residents in facilities for the elderly. The elderly living in facilities usually present with more severe pneumonia and 80% of them eventually die (i.e., only 20% can survive). Osler (1898) declared 100 years ago "Pneumonia is friend of the elderly." This statement is true even today; no progress has been made. By contrast, daily oral care decreased the incidence of aspiration pneumonia and resulted in a reduction of mortality by 50% (Yoneyama et al. 2002). Oral care was generally proven to be superior to modern therapeutic antibiotics. The study suggested that elderly patients without teeth also required the same level of oral care as those with teeth.

Aspiration of gastric juice causes pneumonia that is three times greater in severity (Ohrui et al. 1997). How can the elderly prevent aspiration of gastric juice? Simply let them sit up after a meal, so that gravity prevents their aspirations, even when they have gastroesophageal reflux (Matsui et al. 2002). Our work indicated that this method reduced the incidence of aspiration pneumonia to one-third.

Feeding tube

The final decision is whether or not to feed them by mouth (Nakajoh et al. 2000). The elderly with incoherent responses for at least half a year or almost no appetite, are considered towards the end of their life in the US or European countries. In Japan, 10% of them are considered as dying, and 90% of them survive on average one more year by alimentation therapy (Kosaka et al. 2000). When we asked the caregivers about forced alimentation, 60% of people answered that their patients accepted it because they did not have a choice (Kosaka et al. 2003). Meanwhile, 90% of the caregivers responded that they did not wish this to be performed for themselves or their parents. Japanese people are often easily influenced by the opinions of others. When the patients were told, "everybody said they do not want to do it," then forced alimentation therapy reduced by half. To discontinue nutrition immediately after the elderly become bedridden, as in the US, was not appropriate, but prolonging the life for a year or more, was not appropriate either. A compromise between both approaches has become generally accepted in Japan.

Immunity

The elderly commonly suffer from infections with unknown origin, such as pneumonia with Mycobacterium mavium-intracellulare complex, because of their immunocompromised status, namely, deficiency of cellular immunity against bacterial infection (Ebihara and Sasaki 2002a). They are not exposed to bacteria every day, which reduces their immunologic competence. Some of the more active elderly people hurt themselves by minor injuries, which increases their immunity. Too much cleanliness decreases their immunity, as infection with a BCG vaccine enhances immunity (Ohrui et al. 2000). A PPD test is the best way to investigate cellular immunity (Nakayama et al. 2000). Elderly Japanese who are more than 65 years old should be positive for PPD, whereas if they are immunocompromised, it changes to negative. BCG vaccinated people who are positive for PPD, have elevated immunity that is prophylactic for pneumonia (Nakayama et al. 2002).

Influenza vaccination is routine for those who need in-home care or are residents in facilities (Fukushima et al. 1999a). Data from our own work contributed to establish this guideline (Fukushima et al. 1999b). Furthermore, Pneumococcus contributes to 30% of community-acquired pneumonia. Pneumovax against Pneumococcus is effective for those who need in-

home care as well. Only 1% of the elderly receive the vaccine in Japan, whereas, 58% of the elderly are given the vaccine in the US and European countries. The US government plans to raise this number to 90%. This plan should be implemented to the same degree in Japan as well.

Pulmonary disorders

Pulmonary functions including vital capacity, which generates energy for living, decrease with advancing age (Nakamura et al. 2002). The vital capacity declines until it becomes difficult to live, in 100 year-old people, even those who are nonsmokers. All organs in their body decrease in function (Ohrui et al. 2004a). The Ministry of Health, Labour and Welfare in Japan has stated that statistically almost all of the 100 year-old people suffer from dementia. Cardiac or kidney functions also decline in these people. These elderly must accept their fate with resignation. Men seem more powerful than women, but yet they generally die seven years earlier than women. Only few men are able to survive longer. By contrast, if women have a long life, their physical performances generally appear lower.

The aforementioned statistics deal with nonsmokers. Smokers have an even shorter life (Suzuki et al. 2001a). They never refrained from smoking. In addition to the damage they do to themselves directly, secondhand smoke is even more environmentally toxic. This is worrisome especially for the children. Thirty percent of children of tobacco smoking parents exhibit a tendency to develop asthma that is common worldwide. However, the results from our study demonstrated that the incidences of asthma are the same in the children of smoking or nonsmoking parents (Ohara et al. 2002). This is likely because 70% of Japanese smoker parents do not smoke at their home, and more than 99% of smoker parents do not smoke when they are with their children. Since they have learned that abstaining from smoking does not harm their children, they should quit smoking when they are with other children or even in public.

Leukotriene receptor antagonists are used as treatment for asthma. Although they are not so efficacious, they work occasionally during sleep (Kanda et al. 2000). This suggests that the allergic reaction and activity of cranial nerves in which allergic reactions take place are important. Both of these are indispensable to the development of an asthmatic attack. One of the conditions resulting in the most significant decrease in function of the cranial nerves is Alzheimer's disease. Asthma never manifests, if the cranial nerves, where the allergic reactions occur, exhibit lower activity, even if airway hypersensitivity or IgE allergy is evident (Ohrui et al. 2002). Thus, asthma does not develop in patients with Alzheimer's disease.

Alzheimer's disease

One of the objective diagnostic methods for Alzheimer's disease is to detect the presence of phosphorylated Tau in cerebrospinal fluid (CSF) that occurs in 85% (Itoh et al. 2001). Following objective diagnosis of Alzheimer's disease, how will it be possible to treat them? Oral care to stimulate their cognition might improve only one of 30 points in MMC score. Aricept is not so effective. One of the Chinese medicines, kamiuntanto®, is effective, but is equivalent to Aricept®. Combinations of these medicines are slightly more effective, but generally last only half a year (Suzuki et al. 2001b). However, the ACE inhibitor, Coversyl®, has been shown to decrease Alzheimer's disease to one-quarter (Ohrui et al. 2004b). Some of the ACE inhibitors penetrate the blood-brain barrier (BBB) and increase substance P that stimulates neutral endopeptidase (NEP), which degrades substance P (Ohrui et al. 2004c). However, this NEP has also been shown to resolve amyloid β-protein. Dr. Saido in Japan described the biochemical pathway by which NEP can catabolize amyloid β-protein in Nature Medicine in 2002 (Iwata et al. 2000). We found a prophylactic treatment for Alzheimer's disease on the basis of their data. We administered Coversyl® to the patients with Alzheimer's disease and decreased only one point in the MMC score after a year, whereas a Ca^{2+}-antagonist decreased it by four points (Ohrui et al. 2004d). Today, the newest treatment for Alzheimer's disease is vaccine therapy against amyloid β-protein.

However, 6% of these patients may develop nonbacterial meningitis (Hock et al. 2003). Therefore, we believe that other treatments, such as Coversyl®, would be more efficacious.

However, Alzheimer's disease does not resolve by simply ceasing the decline in cognitive function. Behavior disorders are also to be noted in these patients, which occasionally manifest as violation or vocal abuse. Consequently, if Gramalil® or a major tranquilizer is prescribed, it can cause reduced dopamine levels that can result in aspiration or inability to walk. When we prescribed the Chinese medicine, Yokukansan®, behavior disorder and activities of daily living (ADL) improved in these patients.

Depressive state

The elderly often suffer from depression, which may be due to social or familial problems (Shinkawa et al. 2002a). The depression causes the elderly to experience a common cold three times more frequently (Shinkawa et al. 2000). Decreased humoral as well as cellular immunity renders them susceptible to common colds or even cancers, as some data have demonstrated. Older patients with limited ADLs are most susceptible to the complications of influenza or common cold, resulting in severe dehydration, heart failure, or secondary bacterial pneumonia. Therefore, it is important to examine the condition of immunity in disabled older people according to their emotional state. We examined the emotional state of disabled older people with the Geriatric Depression Scale (GDS) and immune conditions by antibody responses to influenza vaccination and delayed-type hypersensitivity (DTH) responses to tuberculin, as humoral and cellular immunoreactivities, respectively (Shinkawa et al. 2002b). The rates of positive antibody response and positive tuberculin response of depressed and nondepressed patients were compared. Our results showed that disabled, depressed older people have reduced reactivity in humoral and cellular immunity (Table1). One cause of their depression is their ever-increasing medical expenses. Three children have to take care of one elderly parent. Some of the elderly feel that they do not want to bother their children or are embarrassed that they are living longer. However, to live longer means less medical expenses (Nakajoh et al. 1999). Furthermore, the historical and present-day working populations are almost the same - approximately 50% (Sasaki et al. 1996). We recommend these people to live longer and tell them "It is nothing to worry about."

We investigated the period for living since these elderly patients became bedridden (Kosaka et al. 1998). If they are bedridden after 80 years of age, they will pass away within a year. If this occurs after 90 years of age, they will pass away even sooner. If they are bedridden before 80 years of age, they will likely live for a couple of years. In-home care is most expensive. If you employ in-home caregivers for 12 hours a day, it would cost 10,000 yen. If you also need them during the night, an additional 12 hours will cost a total of 20,000 yen. This would cost 600,000

TABLE 1. *Depressive state and immunoreactivities*

Group	Increased Influenza antibody titers			Positive tuberculin response
	New Caledonia strain	Panama strain	Yamanashi strain	
Non-depressed ($n = 18$)	66.7%	55.6%	50.0%	88.9%
Depressed ($n = 28$)	14.3%	21.4%	17.9%	53.6%
Odds ratio	12.0 (95% CI3.1-45.7)	4.6 (95% CI1.3-16.1)	5.2 (95% CI1.4-19.1)	6.9 (95% CI1.5-31.8)

yen in a month. Moreover, if you add medical expenses to these costs, the total would be 1,000,000 yen in a month, and even 10,000,000 yen in a year. Thus, medical care staff and home caregivers should try to not confine these patients to their beds. It would be more prudent to encourage these patients to consult at the clinic and to start treatment at an earlier stage. The elderly pay only 20,000 yen a month on outpatient bills that would be much less expensive than that of in-home care. Avoiding being bedridden is essential in geriatric medicine.

Frail elderly

It is necessary to learn from the 8% who need in-home care, as to why they become confined to the bed, and to use this knowledge to treat a patient properly, to not become bedridden. This is a key principal in geriatric medicine and care. These data suggest that those persons who are devoted to other people have less serious disease occurrence. Those persons living longer could be called selected people (Katsumata et al. 1995; Kobayashi et al. 1997). The Japanese have the longest life span in the world that indicates having integrated an outstanding culture. In order to increase the average life span by one year, it takes five years (Sasaki et al. 1997). In the US, the life span is five years shorter than that in Japan, indicating that they are behind us by 5 times 5 or 25 years.

There are one million people who are confined to the bed in Japan, meanwhile there are no data representing people who are confined to the bed in the US. However, when we have been to the US to inspect, we have seen several people who are confined to the bed. The individuals requiring in-home care in the US are twice that in Japan, despite a lower number of the elderly in the US (Ebihara et al. 2002b). The Japanese society is affectionate to the elderly, while the American and European society would be cold to the elderly. If we develop a society which has a cold attitude to the elderly as in the US and European countries, the expenditures of elderly care insurance would double, requiring an additional 5.5 trillion yen. It is impossible to cover all these costs. To cut down these expenditures for the elderly, they need to be given adequate status in the society (Ohrui et al. 2004e).

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INTERNATIONAL JOURNAL OF GERIATRIC PSYCHIATRY
Int J Geriatr Psychiatry 2004; **19**: 727–733.
Published online in Wiley InterScience (www.interscience.wiley.com). **DOI**: 10.1002/gps.1161

Smell identification test as an indicator for cognitive impairment in Alzheimer's disease

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SUMMARY

Objectives The aim of the present study was to assess olfactory dysfunction in patients with Alzheimer's disease (AD) and to compare utility of the olfactory tests as possible clinical markers.
Methods Two olfactory identification tests (The Cross-Cultural Smell Identification Test [CC-SIT] and the Picture-based Smell Identification Test [P-SIT]) and the Mini Mental State Examination (MMSE) were administered to patients with AD and age-matched controls. Apolipoprotein E (Apo E) genotypes of patients with AD were identified.
Results Patients with AD had significantly lower olfactory identification scores than age-matched non-demented elderly subjects in both olfactory assessments. In the AD group, the coefficient of correlation between the MMSE scores and the P-SIT scores was higher than that between the MMSE scores and the CC-SIT scores. Receiver operating curve (ROC) analyses for both tests indicated that the P-SIT discriminated AD patients from controls more reliably than did the CC-SIT. Within AD patients, those who were carrying one or two ApoE $\varepsilon 4$ alleles had a higher coefficient of correlation between the MMSE scores and the P-SIT scores than patients without the ApoE $\varepsilon 4$ allele.
Conclusions The results suggest that a short and simple non-lexical olfactory identification test can be useful as a clinical marker of AD appropriate for Japanese elderly population. Copyright © 2004 John Wiley & Sons, Ltd.

KEY WORDS — Alzheimer's disease; impaired olfactory identification; smell identification test; Apolipoprotein E genotypes

INTRODUCTION

Although elderly patients with Alzheimer's disease (AD) are steadily increasing and strategies to cope with various symptoms accompanied by the disease are drawing major concern from clinicians, the biological basis underlying the disease is not yet sufficiently understood. In terms of understanding the neurophysiological basis of the disease and efficacy of therapeutic intervention, it is imperative to establish biological markers with higher sensitivity and specificity by which patients would benefit from early therapeutic intervention (Cummings *et al.*, 2000). Whether preclinical AD can be screened assuredly by existing examinations remains controversial even though various possible biological markers or psychometric instruments have been proposed in recent years (Corder *et al.*, 1993; Minoshima *et al.*, 1997; Bondi *et al.*, 1999; Bookheimer *et al.*, 2000; Goldman *et al.*, 2001; Itoh *et al.*, 2001). A wealth of studies suggest olfactory dysfunction, in particular impairment of olfactory identification, exists in patients with AD (Morgan *et al.*, 1995; Mesholam *et al.*, 1998; Murphy, 1999). Furthermore, the dysfunction may occur at very early stage of the disease, prior to the advent of typical cognitive and behavioral disturbances, hence, assessment of olfactory function can be used as a predictor of the onset of the disease (Nordin and Murphy, 1996; Bacon *et al.*, 1998;

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Contact/grant sponsor: Mitsui-Sumitomo Marine Welfare Foundation.

Received 5 May 2003
Accepted 19 April 2004

Graves *et al.*, 1999; Devanand *et al.*, 2000), as well as being a useful adjunctive screening measure in discriminating AD from other neuropsychiatric conditions of elderly patients (Solomon *et al.*, 1998).

In terms of onset of the disease, apolipoprotein E genotype has been widely acknowledged as a factor that can affect the onset of clinical manifestation (Noguchi *et al.*, 1993). The presence of one or more apolipoprotein E ε4 allele has been reported to increase the risk of cognitive decline in older adults with unexplained olfactory dysfunction (Murphy *et al.*, 1998; Borenstein Graves *et al.*, 1999). Volumetric MRI measurements of early AD patients have shown that apolipoprotein E ε4 allele is associated with the degree of atrophy in the entorhinal cortex, which receives direct sensory projection from the olfactory bulb (Juottonen *et al.*, 1998; Insausti *et al.*, 2002), and also robust relationships between mesial temporal lobe volumes and olfactory functional measures have been confirmed (Murphy *et al.*, 2003). These findings suggest a possible interaction of apolipoprotein E genotype with olfactory performance in AD patients.

In assessing olfaction in humans, various clinical tests of smell have been proposed in previous studies, most of which were aimed to assess ability to either detect smells or identify them (Cain and Gent, 1991; Doty *et al.*, 1996). Olfactory identification is believed to involve higher processing of olfactory stimuli than just detecting smells (Tanabe *et al.*, 1975). Previous studies on olfaction in patients with AD have suggested a possibility that a decline in olfactory identification may precede that of olfactory detection threshold (Serby *et al.*, 1991). Considering the influence of cultural background and personal history in identifying odors, clinical tests of smell applied in previous studies may not have been adequate in assessing olfaction of Japanese elderly population. Also, olfactory tests that rely on lexical function may not directly reflect olfactory dysfunction in patients with dementia (Morgan *et al.*, 1995). Thus, a necessity to generate a battery of standardized olfactory assessment, capable of bypassing cultural bias, emerges (Parola and Liberini, 1999).

In this study, we investigated olfactory identification in patients with AD and age-matched non-demented elderly subjects using two different olfactory identification tests (lexical and non-lexical), and examined the correlation between the degree of cognitive decline and the scores of the two tests with an aim to assess relevance of the tests for clinical application. We also examined a possible interaction of Apo E phenotype with patients' performance in olfactory identification.

SUBJECTS AND METHODS

We administered two olfactory tests to 85 subjects (mean age $\pm$ SD: 76.3 $\pm$ 7.2) who met Diagnostic and Statistical Manual of Mental Disorders, Fourth Edition (DSM-IV) and National Institute of Neurological and Communicative Disorders and Stroke-Alzheimer's Disease and Related Disorders Association (NINCDS-ADRDA) criteria (MacKhann *et al.*, 1984) for AD, and 30 age-matched (mean age $\pm$ SD: 74.8 $\pm$ 8.5) non-demented elderly subjects. All subjects, who were screened to exclude conditions affecting olfactory function, (e.g. smoking, history of head trauma or nasal disease, presence of respiratory infection, metabolic disorders, medication or cognitive decline), were recruited from the Memory Impairment Clinic at Nagoya University Hospital. Written informed consent was obtained after complete description of the study.

All subjects were subjected to cognitive assessment using the MMSE (Folstein *et al.*, 1983). In order to examine the interaction of olfactory ability with Apo E status, blood samples of patients with Alzheimer's disease were taken for Apo E genotyping. The Apo E genotypes were analyzed and identified using the polymerase chain reaction (PCR) and Hha I digestion as described elsewhere (Emi *et al.*, 1988; Hixon and Vernier, 1990). Digested DNA fragments were separated with polyacrylamide gel electrophoresis and separated fragments of DNA were visualized using ethidium bromide staining.

Two tests for assessing olfactory identification were administered to all subjects. One was the Cross-Cultural Smell Identification test (CC-SIT), a widely used test of odor identification involving a scratch-and-sniff test of 12 microencapsulated odorants with a forced choice of four alternatives per item (Doty *et al.*, 1984a). The other test was picture-based smell identification test (P-SIT), in which subjects were asked to smell six distinctive odorants (ground coffee, incense, ground sesame, green tea, fermented soy-bean paste and soap) that were confirmed to be intense and familiar to cognitively intact Japanese elderly population by preliminary trials. The subjects were instructed to inhale the odorants with their eyes closed through both nostrils without sniffing. Twenty pictures of materials including six pictures corresponding to the smells and fourteen pictures of the materials unrelated to the odorants were presented, and the subjects were asked to identify the odorants by choosing a corresponding picture after smelling each odorant. Before both trials, subjects were confirmed to be able to either understand words presented

for choice or identify all the materials of the pictures, and those who could not answer correctly were eliminated from the study. Each trial was carried out with an interval of 30 seconds in order to avoid the interaction with odors previously presented. The number of correct choices was recorded as a score of the test.

Means age over the two study groups (elderly controls and AD group) were compared by Student's *t*-test. Gender difference of MMSE scores within each group, mean MMSE scores of patients with the $\varepsilon 4$ ($\varepsilon 4+$) allele and without the $\varepsilon 4$ ($\varepsilon 4-$) allele, mean P-SIT and CC-SIT scores of the two groups were compared by Mann–Whitney's U-test. Differences between means were considered statistically significant if $p < 0.05$. The relationship between the age of the subjects and the scores of the P-SIT and the CC-SIT in both groups, and the relationship between the MMSE scores and the scores of the two olfactory tests within the AD group and when divided by two ApoE phenotypes ($\varepsilon 4+$ and $\varepsilon 4-$) were examined using Spearman rank of order correlation coefficients. We also conducted simple regression analyses for both olfactory tests within the AD group and for the P-SIT when AD patients were divided by two ApoE phenotypes to calculate the predicted MMSE scores from the SIT scores as independent variables and compared coefficients of determination. In order to compare the validity of the smell identification tests in discriminating AD group from elderly controls, we carried out receiver operating curve (ROC) analysis at different cut-off scores in each test.

All statistical analyses were performed on a personal computer with the statistical package SPSS for Windows (Version 11.0; SPSS Inc., Chicago, IL, USA).

RESULTS

The two groups did not differ in terms of age ($p = 0.38$). Mean MMSE score of the AD group (mean ± SD: 19.6 ± 4.6) was significantly lower than that of the elderly controls (mean ± SD: 28.5 ± 2.2) ($p < 0.0001$) (Table 1). Within the AD group, there were no gender differences in age ($p = 0.18$), MMSE scores ($p = 0.93$) and in the scores of the two olfactory identification tests (P-SIT; $p = 0.68$, CC-SIT; $p = 0.23$). The ApoE genotypes for patients with AD were: $\varepsilon 23$, $n = 4$; $\varepsilon 33$, $n = 27$; $\varepsilon 24$, $n = 3$; $\varepsilon 34$, $n = 38$; $\varepsilon 44$, $n = 4$. Patients who were carrying one or two $\varepsilon 4$ alleles did differ in age ($p < 0.001$) but did not differ in the MMSE score ($p = 0.30$) and in the scores of the two olfactory identification tests (P-SIT: $p = 0.84$, CC-SIT: $p = 0.93$) from those without the $\varepsilon 4$ allele. All subjects (AD patients and elderly controls) were confirmed to correctly identify all the pictures of the materials in the P-SIT. However, many participants responded while administering the CC-SIT that it was difficult to imagine the corresponding odors of some of the alternatives such as turpentine or paint thinner.

Table 1. Demographic variables of participants

	Alzheimer's ($n = 85$)	Non-demented ($n = 30$)
Mean age ± SD	76.3 ± 7.2	74.8 ± 8.5
Age range (M/F)	58–89 (17/68)	60–94 (12/18)
Student's *t*-test	$p = 0.38$, $t = 0.89$, effect size = 0.20	
Mean MMSE score ± SD	19.6 ± 4.6	28.5 ± 2.2
Score range	12–28	24–30
Mann–Whitney's U Test	$p < 0.0001$, $z = 4.76$, effect size = 1.71	

A significant difference of the P-SIT scores between the AD group (mean ± SD: 1.5 ± 1.3) and the elderly controls (mean ± SD: 4.4 ± 1.2) was observed ($p < 0.0001$, $z = 6.848$). Difference of the CC-SIT scores between the two groups also reached a statistical significance (AD group; mean ± SD: 4.1 ± 2.5, elderly controls; mean ± SD: 6.8 ± 1.7), ($p < 0.0001$, $z = 4.339$) (Figure 1). Effect size of difference between the two groups for the P-SIT (1.59) was greater than that of the CC-SIT (1.04).

Regarding the effect of age on the performance of olfactory tests, negative correlation was observed between the age and the score of the P-SIT both in the elderly controls ($\rho = -0.77$, $p < 0.001$) and in the AD group ($\rho = -0.23$, $p = 0.045$). Meanwhile, correlations between the age of the subjects and the CC-SIT scores in both groups did not reach a level of statistical significance (elderly controls:

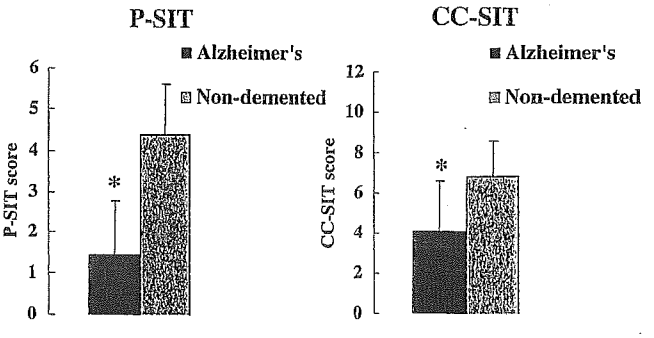

Figure 1. Comparison of the Smell Identification Test (P-SIT and CC-SIT) scores of patients with Alzheimer's disease and non-demented elderly subjects. *$p < 0.0001$

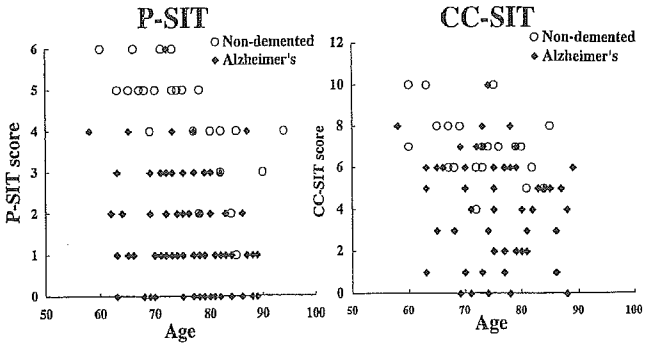

Figure 2. Correlations between age and Smell Identification Test (P-SIT, CC-SIT) scores in the non-demented elderly subjects (open circle) and in patients with Alzheimer's disease (closed square)

$\rho = -0.34$, $p = 0.13$; AD group: $\rho = -0.18$, $p = 0.18$) (Figure 2).

Correlations of the MMSE scores with the two olfactory test scores in the AD group are compared in Figure 3. The coefficient of correlation between the MMSE scores and the scores of the P-SIT was higher ($\rho = 0.57$, $p < 0.001$) than that between the MMSE scores and the scores of the CC-SIT ($\rho = 0.37$, $p < 0.01$) (Figure 3). Simple regression analyses indicated that the P-SIT score predicted MMSE score with a higher coefficient of determination (adjusted $R^2 = 0.33$) than did the CC-SIT (adjusted $R^2 = 0.11$).

ROC analyses for both olfactory tests indicated that the P-SIT discriminated AD group from elderly controls with higher sensitivity and specificity than the CC-SIT in varying cut-off scores. Az (area under the ROC curve) values for both tests were 0.993 (P-SIT) and 0.878 (CC-SIT) respectively (Figure 4). Based on the results of the ROC analyses, optimal cut-off scores for both tests (P-SIT: < or = 3, CC-SIT: < or = 5) were chosen for chi square analysis.

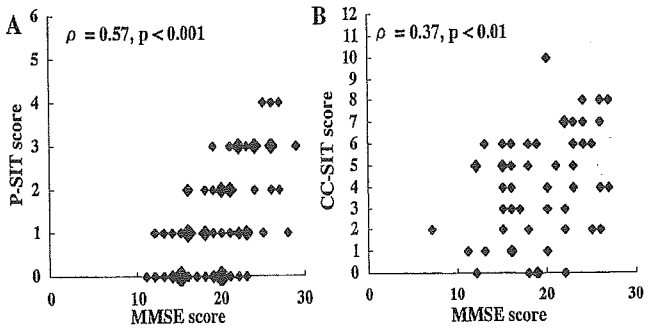

Figure 3. Scatterplots demonstrating relationship between MMSE score and (A) Picture-based Smell Identification Test (P-SIT) score and (B) Cross-Cultural Smell Identification Test (P-SIT) score and (B) Cross-Cultural Smell Identification Test (CC-SIT) score in the AD group. Range of scores: P-SIT: 0–4; CC-SIT: 0–10. Higher scores reflect better olfactory identification

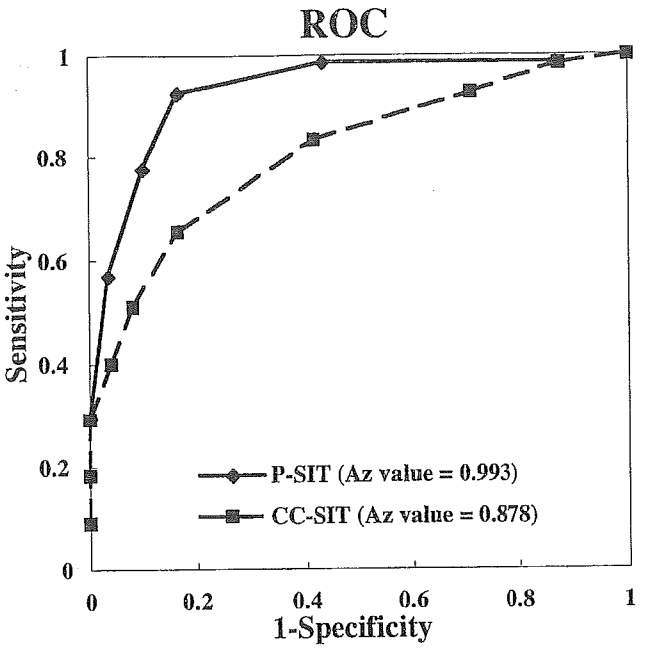

Figure 4. Receiver operating curve (ROC) analyses of the smell identification tests (P-SIT and CC-SIT) for discriminating AD patients from elderly controls

The result showed that the P-SIT discriminated AD group from elderly controls with high classification parameters (sensitivity; 0.94, specificity; 0.81, positive predictive value; 0.93, negative predictive value; 0.83, $p(\chi_0^2) < 0.0001$). Meanwhile, those parameters were lower in the CC-SIT (sensitivity; 0.90, specificity; 0.51, positive predictive value; 0.65, negative predictive value; 0.83, $p(\chi_0^2) < 0.001$).

To test whether ApoE genotype affects the observed correlation between cognitive performance and olfactory identification, we further examined correlations between the MMSE scores and the scores of the P-SIT depending on ApoE phenotypes. When dividing patients with AD according to the ApoE $\varepsilon 4$ allele, patients who were carrying the ApoE $\varepsilon 4$ allele had a higher coefficient of correlation between the MMSE scores and the scores of the P-SIT ($\rho = 0.67$) than that of patients without the ApoE $\varepsilon 4$ allele ($\rho = 0.46$). Simple regression analyses to calculate the predicted MMSE score from the P-SIT score indicated that in patients with the ApoE $\varepsilon 4$ allele, coefficient of determination was higher (adjusted $R^2 = 0.42$) than that of patients without the ApoE $\varepsilon 4$ allele (adjusted $R^2 = 0.18$).

DISCUSSION

Unlike other sensory modalities such as vision or hearing, dysfunction in olfaction may not be well

recognized as an objective symptom in clinical settings despite patient's subjective complaints or substantial impact on mood and social behavior (Kirk-Smith and Booth, 1987). Age-related decline in olfaction has been reported elsewhere (Doty *et al.*, 1984b; Schiffman, 1997). Phylogenetically, sense of smell developed early in mammalian animals and olfactory pathway within the brain has privileged access to major parts of the limbic areas associated with mnemonic function (Eslinger *et al.*, 1982; Eichenbaum *et al.*, 1996). Neuropathologic evidence suggests the involvement of regions related to olfactory processing in the pathogenesis typical of AD revealed by disproportionate numbers of neurofibrillary tangles and neuritic plaques relative to other sensory pathways (Reyes *et al.*, 1987; Reyes *et al.*, 1993). Therefore, a question arises about which level in olfactory processing explains the olfactory dysfunction observed in this study. The results of olfactory tests showing that disability in identifying smells precedes that in smell detection (Serby *et al.*, 1991; Morgan *et al.*, 1995) and neuropathologic findings suggesting the involvement of regions essential for olfactory processing (Hyman *et al.*, 1984; Pearson *et al.*, 1985; Hock *et al.*, 1998) may imply a central origin of olfactory dysfunction rather than peripheral alteration in AD. Although our study did not examine odor detection threshold, we confirmed throughout the two olfactory tests that all subjects detected the presence of odorants administered, which implies that the deficit in odor identification was not due to an inability to detect smells, but rather may have been caused by an alteration in higher olfactory processing, although the trigeminal effect cannot be excluded in interpreting the results.

There was a strong negative correlation ($\rho = -0.77$) between the age and the scores of the P-SIT in the elderly controls, which is in line with the results of a previous report showing that odor identification declines with age (Doty *et al.*, 1984b). However an age-related decline of odor identification scores in the non-demented elderly subjects was not evident when using CC-SIT ($\rho = -0.34$) as compared to using P-SIT, which raises a question about whether the test can be a relevant test battery for assessing olfaction in Japanese elderly subjects. Weak intensity of the smells, as many of the participants reported, may contribute to the poor correlation between the scores of the CC-SIT and the MMSE ($\rho = 0.37$) than that between the scores of the P-SIT and the MMSE scores ($\rho = 0.57$) in the AD group. However, it is unlikely that the lower intensity of the odorants used in the CC-SIT alone can account for the discrepancy of the results between the two tests. As observed from the responses of the subjects participated, some of the odorants such as turpentine or paint thinner included in the CC-SIT were not always familiar to Japanese elderly population, while all the odorants used in the P-SIT were equally familiar to all the subjects. Thus the difference in the intensity and the familiarity of the odorants chosen may have brought inconsistent results regarding correlation between the degree of cognitive impairment and the score of the tests.

We confirmed that non-lexical based test of odor identification could reliably discriminate well-defined AD patients from non-demented elderly subjects with high sensitivity and specificity. These results suggest a possible utility of assessing olfactory identification in differentiating AD patients from subjects with other medical conditions, although olfactory dysfunction is not specific to AD. Pathological evidences and observed correlation between the degree of impairment in olfactory identification and that of cognitive impairment in AD patients lead us to believe that the olfactory dysfunction may occur before cognitive symptoms manifest (Nordin and Murphy, 1996; Devanand *et al.*, 2000), which may give olfactory assessment a potential role as a predictor of the onset of the disease. A community-based cohort study showed that impaired olfaction on CC-SIT increased the odds for cognitive decline, which was verified by a global cognitive test (Graves *et al.*, 1999). Also, a study of patients with mild cognitive impairment demonstrated that olfactory deficits, accompanied by lack of awareness of the problem, was a reliable predictor for development of Alzheimer's disease (Devanand *et al.*, 2000). The results of these studies suggest a potential significance of olfactory dysfunction as a preclinical marker of AD, although we have to bear in mind that rigorous control of medical history and conditions affecting olfaction in sampled population may be crucial in assessing olfactory impairment specifically related to neurodegenerative disorders in elderly patients. Considering cross-sectional aspects of this study, the results do not support advantages of smell identification test over standard psychometric or neuropsychological measures for early detection of AD. Longitudinal follow-up of subjects with impaired smell identification may help examine the utility of smell identification test as a useful screening method for AD.

The strong correlation between the MMSE scores and the P-SIT scores in AD patients who were carrying one or two $\varepsilon 4$ alleles relative to those without $\varepsilon 4$ alleles is intriguing. The finding may suggest a