(54) Title: DIAGNOSTIC TEST FOR ALZHEIMER'S DISEASE

(57) Abstract

Persons suspected of being afflicted with Alzheimer's disease or related diseases can be diagnosed by application of a match-to-sample olfactory test using a panel of unfamiliar odors. Persons afflicted with these diseases are unable to match unfamiliar odors, whereas normal individuals are invariably capable of doing so with a high degree of accuracy.
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DIAGNOSTIC TEST FOR ALZHEIMER'S DISEASE

Government Support

This invention was made with government support under Contract No. N00014-84-K-0391 with the Office of Naval Research and the University of California. The government has certain rights in this invention.

Technical Field

The invention relates to the diagnosis of diseases effecting the brain, in particular Alzheimer's disease. Specifically, the invention relates to a simple noninvasive test to diagnose Alzheimer's or similar brain dysfunctions.

Background Art

Alzheimer's disease is relatively recently recognized as a major social and economic problem among an aging population group. The progression of the disease is associated with a loss of cognitive function which is reflected in actual physical cellular destruction and disorientation in the brain tissue. These pathologies have been localized to the subiculum and superficial layers of the entorhinal cortex (Hyman, B.T., et al. Science (1984) 225:1168-1170; Pearson, R.C.A., et al. Proc Natl Acad Sci (USA) (1985) 82:4531-4534; Ball, M.J., et al. Lancet (1985) Jan 5:14-16. Indeed, the anatomical changes associated with Alzheimer's disease have been shown to occur in nondemented subjects using data obtained on autopsy, suggesting that these anatomical changes proceed in the entorhinal cortex before the symptoms of the disease become apparent (Ullrich, J., Anals Neurol (1985) 17:273-277). While it is known that the lateral
portions of the entorhinal cortex are monosynaptically innervated by the lateral olfactory tract in mammals, a reliable diagnostic test based on olfaction which permits diagnosis of early stages of the disease or which permits assignment of behavioral symptomologies to the anatomical changes associated with Alzheimer's has not been devised, although attempts have been made.

All of these attempts utilize the ability or inability of the subject to recognize familiar odors. Serby, M., et al. Am J Psychiatry (1985) 142:781-782 found that Alzheimer's patients and patients with Parkinson's disease were unable to identify, for example, catsup, by smell, in comparison with the odor of mint when asked to do so. Peabody, C.A., et al. Am J Psychiatry (1985) 142:524-525 found that persons with dementia associated with Alzheimer's were unable to identify lemon, peppermint, coffee, maple and vinegar correctly, some because they could not perceive the odors at all and some because they were confused about them. Walk, H.A., et al. Perception and Psychophysics (1984) 36:508-514 showed that the memory code for odors may incorporate semantic information; the odors tested were familiar ones. Richard. J., et al. Acta Neurol Belg (1981) 81:333-351 showed that a diminution in olfactory capacity was associated with dementia and suggested that olfactory capacity might be a criterion on which to base diagnosis.

It is already understood that the anterior temporal region of the brain is associated with olfactory information processing, and this has been established by examining the olfactory capacities of patients who have undergone temporal lobotomies as treatment for epilepsy (Eskenazi, B., et al. Neurol Psychologia (1983) 21:365-374; Rausch, R., et al, Cortex
These regions of the brain, however, are not believed to be implicated in the common dementias associated with Alzheimer's or Parkinson's disease.

The recognition of familiar odors, of course, must rely on a number of factors other than the olfactory system per se, for example, long-term memory, semantic abilities, and associative powers. Since the association of olfaction with Alzheimer's disease is believed to reside directly in disruption of the cellular network associated with the olfactory circuit per se, a diagnosis based on dysfunction of this particular region would have superior diagnostic qualities.

Tests similar to those of the invention have been used in animals as indicators of damage to the entorhinal cortex (Staubli et al., Proc Natl Acad Sci (USA) (1984) 81:5885-5887). Rats having electrolytic lesions in the lateral entorhinal cortex, which is innervated by the lateral olfactory tract, lost their ability to "remember" a particular odor after 3-10 minutes.

Disclosure of the Invention

The invention provides a diagnosis for memory dysfunction which is associated with those areas of the brain affected by Alzheimer's and Parkinson's disease, specifically the entorhinal cortex. The diagnosis is based on a short-term memory test which assumes no prior knowledge of the materials being tested and a minimum of or no verbal skills.

The diagnostic method of the invention is based on the subject's ability to recognize the repetition of an immediately precedent experience with an unfamiliar
odor. Because no verbal skills are required, the test can be adapted to animal studies and used as a criterion for efficacy of factors relating to analogous dementias in animals, thus providing a model system to study these diseases and their treatments. The subject is presented with a panel of odors which are ordinarily not experienced by individuals (except perhaps those in specialized walks of life). Appropriate choices for such odors are ingredients used in the perfume industry, none of which have been individually experienced by the general population. The subject is presented a sample smell from this panel and then asked to identify which of three successively presented test odors it matches. In general, the test comprises the recognition or nonrecognition of an immediately precedent unfamiliar odor among a selection of several choices of unfamiliar odors.

Further, proposed treatments can be evaluated with respect to their effectiveness by noting whether or not the treatment improves the performance of the subject in the test. Since the test can be designed for use in animals, they can be employed as model systems to test treatment methods.

Therefore, in one aspect, the invention relates to a method for diagnosing Alzheimer's disease or other short-term memory deficiencies involving the same circuitry which comprises:

a) causing a mammalian subject to smell a specific odor, which odor is unfamiliar to the subject;

b) immediately thereafter causing the subject to smell each of a series of at least two unfamiliar odors, which series includes the odor of a) above; and
c) ascertaining whether or not the subject can correctly identify the previously smelled odor among the series presented in b).

The invention also relates to kits prepared to administer the diagnostic test of the invention, and to methods to evaluate treatment protocols by employing those tests.

**Brief Description of the Drawings**

Figure 1 shows a comparison of the results of tests for dementia using familiar as compared to unfamiliar odors.

Figure 2 shows a test card suitable for administering one embodiment of the test of the invention to a particular individual.

**Modes of Carrying Out the Invention**

The invention relies on the determination of the ability or inability of the subject to "learn" to recognize an unfamiliar odor as evidenced by the ability or inability to select that odor from a series after immediate previous exposure to the unfamiliar odor. The alternate choices provided to the subject will also be unfamiliar.

As used herein "unfamiliar" odor refers to an odor to which it is unlikely the subject has been previously exposed. It is really not necessary to conduct extensive tests to decide whether or not by some quirk an individual may have accidentally come across a particular odor in the past. Some materials are so infrequently found in the familiar environment that unless an individual has had a particularly peculiar employment history, the likelihood of previous exposure is practically nil. Used in the illustration below are
components of perfume manufacture which are not generally found individually in the environment. As they exist in manufactured perfumes, their odors are so intermingled that the sense provided by an individual component is entirely unfamiliar to anyone not previously employed as a perfumer. Of course, other such odors could also be used. There are nearly six million known organic compounds, many of which have odors which have been experienced only by the individual synthesizing them. However, individual perfume components is a convenient source of ready supply.

By selecting unfamiliar odors, the subject is provided a fresh opportunity to "learn" the odor and the results of the test do not depend on previous learning, semantic ability and word associations, or long-term memory. The test of the invention, therefore, specifically does not employ such familiar odors as mint, wintergreen, vanilla, cinnamon, and so forth that would be part of the subject's previous experience.

The test is also designed so that the subject must make a choice between a set number of possible candidate odors, only one of which is identical to that previously provided to the subject. All of these odors, too, must be unfamiliar lest the results be biased by the subject's existent or nonexistent prior associations with the alternate candidates. This system also provides a predictable statistical probability of random choice of the correct material, as well as making it unnecessary for the subject to verbalize or otherwise describe the sensation.

In the conduct of the test, the subject is presented with a sample odor and then immediately asked to identify which of three successively presented test odors it matches. Of course, the number of odors in the
selection panel can be varied, but three seems a reasonable number, having a sufficiently low probability (33%) of a random correct choice. Two odors can also be used, and are preferred when the test is adapted to animals. As shown in the example below, the results of this test correlate well with the incidence of Alzheimer's disease when tested in a group of subjects who have previously been diagnosed and compared to normal persons known to be free of this disease. While the illustration below specifically correlates this test with Alzheimer's disease, it is clear that it can also be used to detect the incidence of closely related diseases which affect the same portion of the brain.

The materials which are useful in the invention can also be packaged as kits to permit easy administration of the test. Such kits will contain several materials, at least three, but preferably more than ten materials with unfamiliar odors packaged separately so that these materials can conveniently be provided to the subject. If desired, the materials can be supplied as individual bubbles on a test card so that the administrator of the test can break the bubbles in the correct order from an individual card for each subject. Of course, a variety of configurations are possible, but a workable configuration is shown in Figure 2. The card contains the first odor under the bubble labeled 1; the bubbles of the series to be compared are labeled A, B, and C, only one of which is the same as 1. The card is perforated at the center and between the panel bubbles so that the halves and the panel odors can be supplied separately, avoiding the possibility of mingling the odors. The materials may also be supplied as separate containers, or the administrator of the test can repack these materials.
according to particular protocols. In any event the kit will contain instructions setting forth the protocols and the manner of interpreting the results according to the method of the invention described above.

Characterization of disease states of the type of concern here, such as Alzheimer's disease, is extremely difficult as no single factor (such as an infective organism) can be identified to absolutely classify the cause, as could be done for, for example, measles or influenza. In general the disease is characterized by its symptomology and the pathology of certain tissues which can only be detected upon autopsy. The test herein is diagnostic of particular brain cell disruptions associated with sufferers of Alzheimer's disease. However, it is, of course, possible that the same disruption in conjunction with alternative pathologies in other parts of the brain may result in symptomologies which are similar but not identical to those of Alzheimer's disease. Therefore, the test herein should be regarded as a diagnostic for all diseases which have the same impact on the olfactory system as does Alzheimer's disease. It is not known at present the total scope of such diseases or degenerative states.

In addition to its use to diagnose these degenerative diseases, the method of the invention may also be used to monitor treatment protocols. Treatment protocols applied to human subjects may be directly evaluated by repeatedly testing the subject to whom the treatment is applied according to the subject's performance in the method described. In addition, animal models can be used to evaluate alternative treatments by producing artificial lesions analogous to those characteristic of the disease state and using the
method of the invention to assess the effect of these treatments on performance.

When performing the method of the invention on animals, of course, accommodation must be made according to the inability of the animal to directly communicate its choices. Accordingly, a reward system can be utilized, such as that described by Staubli et al (supra).

In a typical procedure, lesions corresponding to Alzheimer's or related disease are produced in test animals, such as rabbits, rats, or mice, and the animals are evaluated according to the method of the invention for their ability to recall an unfamiliar odor. The animal is then administered the prepared treatment and the animal’s performance assessed. Treatments which result in the improvement of performance are considered promising in the treatment of the disease.

Examples

The following studies illustrate the manner of carrying out the invention and are not to be construed as limiting its scope.

Eleven patients of average age 59.8 years (range: 53 to 66), diagnosed according to NINCDS-ADRDA task force recommendations (McKhann et al, Neurology (1984) 34:939-944), and ten controls matched for age, sex and race were studied. All subjects were non-smokers and had no history of neurological problems, outside of Alzheimer's. The standard olfactory tests known in the art and the invention test were administered on separate days. The results of both tests for the first set of six patients and seven controls are shown in Figure 1.
The Smell Identification Test (SIT; Sensonics Inc., Philadelphia, PA) is a standardized test with well established sex and age norms. It involves a series of trials in which the subject is asked to select one of four printed words to describe a familiar odor (Doty et al., *Physiol Behav* (1984) **32**:489-944). Alzheimer's patients scored significantly worse in this test than the control group (72.1% vs 91.1% correct: \( t = 2.85, p < .01 \)) but well above chance levels (25%).

In the test of the invention fifteen unfamiliar odors used in perfume manufacturing were obtained as candidate odors. The subject was presented a sample of one of these and then asked to identify which of three successively presented test odors it matched. Control subjects had little difficulty with this task (63.9% correct); however, Alzheimer's patients were severely impaired (49.5% correct; \( t = 5.52, p < .001 \) compared to controls) with several subjects approaching chance levels of performance (i.e., 33% correct).

Additional subjects have been tested and continue to be tested. All results to date support the initial findings. Figure 3 shows results of the tests, as applied to 10 controls and 11 patients, which are consistent with those shown in Figure 1.

It will be noted that the subjects were much less impaired on the test (SIT) for familiar odors than they were on the novel odor test (invention). Many of the patients used in the study of the invention were not institutionalized and were classified as being in the early stages of Alzheimer's disease. These patients appear to have only a marginal deficit in detection and recognition of odors learned before the onset of the disease, but have lost the ability to encode new smells.
Claims

1. A method to diagnose Alzheimer's disease in humans, and other diseases in humans which have similar effects on the short-term olfactory memory, which method comprises:
   a) causing a subject to be diagnosed to smell a first unfamiliar odor;
   b) immediately thereafter causing said subject to smell successively each of a panel of at least two unfamiliar odors, one of which is the first unfamiliar odor and the others of which are different unfamiliar odors; and
   c) ascertaining whether or not the subject can select which of the panel odors is the same as the first unfamiliar odor.

2. The method of claim 1 wherein the unfamiliar odors are perfume components.

3. A kit for diagnosing Alzheimer's or related diseases in humans which comprises:
   a) at least two containers containing individual materials having unfamiliar odors; and
   b) a set of instructions for administration of the test according to the method of claim 1.

4. A method for testing the efficacy of a treatment protocol for Alzheimer's disease in humans, and other diseases in humans which have similar effects on the short-term olfactory memory, which comprises administering said treatment to a subject afflicted with lesions corresponding to said disease, followed by:
a) causing a subject to smell a first unfamiliar odor;
b) immediately thereafter causing said subject to smell successively each of a panel of at least two unfamiliar odors, one of which is the first unfamiliar odor and the others of which are different unfamiliar odors; and
c) ascertaining whether or not the subject can select which of the panel odors is the same as the first unfamiliar odor.

5. The method of claim 4 wherein the subject is human.

6. The method of claim 4 wherein the subject is a test animal having said lesions artificially introduced.
Percent correct responses on the Smell Identification Test (SIT) and match-to-sample test for Alzheimer's patients (ALZ, n = 6) and control subjects (CNT, n = 7). The dashed line indicates the level of chance performance for each of the tests. (*=p .01; **=p .001)

FIG. 1
FIG. 3
# INTERNATIONAL SEARCH REPORT

**INTERNATIONAL APPLICATION No:** PCT/US87/01737

## I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) 1

According to International Patent Classification (IPC) or to both National Classification and IPC:

- **IPC (4):** A61B 5/00
- **U.S. Cl.:** 128/630; 122/2; 122/61

## II. FIELDS SEARCHED

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**Minimum Documentation Searched**

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched:  

- Chemical Abstracts and Biosis; Alzheimer and Small, odor or olfactory as keywords.

## III. DOCUMENTS CONSIDERED TO BE RELEVANT 1

<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of Document, 1) with indication, where appropriate, of the relevant passages 1)</th>
<th>Relevant to Claim No. 1)</th>
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### Notes:

- Special categories of cited documents: 13
- "A" document defining the general state of the art which is not considered to be of particular relevance.
- "E" document essential to the invention.
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified).
- "O" document referring to an oral disclosure, use, exhibition or other means.
- "P" document published prior to the international filing date but later than the priority date claimed.
- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention.
- "Y" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step.
- "W" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "X" document member of the same patent family.

## IV. CERTIFICATION

**Date of the Actual Completion of the International Search 1**

13 November 1987

**Date of Mailing of this International Search Report 1**

02 DEC 1987

**International Searching Authority 1**

ISA/US

**Signature of Authorized Office**

[Signature Image]  

Randall E. Snow
FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

Rapid Forgetting of Olfactory Information in Rats", See pages 5885 to 5887.

V. OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. Claim numbers because they relate to subject matter not required to be searched by this Authority, namely:

2. Claim numbers because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

VI. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

This International Searching Authority found multiple inventions in this international application as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant’s protest.

☐ No protest accompanied the payment of additional search fees.