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Cognition-activated low-frequency modulation of light absorption in human brain

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ABSTRACT **Animal model studies indicate light-absorption changes of the exposed animal brain in response to visual stimulation. Here we report observations of red-light absorbancy changes, attributable to repetitive blood concentration changes in response to stimulation in the human brain frontal region by a cognitive process. These responses are observed as low-frequency recurrence of changes by Fourier transform analysis and are attributed to blood concentration change stimulated by the increased metabolic rate of brain tissue in cognitive function. A simple, portable dual wavelength spectrophotometer was attached noninvasively to the human forehead to measure the low frequency and power spectra of fluctuations of absorbancies attributed to variations of brain blood concentration in the frontal region. The responses are associated with brain activity in responses to problem solving of analogies presented visually that require an associative function in the frontal region. The method of subtraction of test − rest Fourier transforms minimizes the arterial pulse frequency contributions and identifies specific frequencies—for example, 0.8, 1.6, 1.8 Hz in 24 of 28 tests of nine individuals (85%). Tests in which no increased brain activity was elicited (rest − rest) showed small differences. It is concluded that low-frequency recurrences of brain activity linked to blood concentration increases can be detected in human subjects with an optical device of potentiality for simplified tests of cognitive function in the 0- to 3-Hz region and with modifications for wider band recordings in localized tissue volumes by time-resolved spectroscopy.**

In this study a simple optical method (1) was used to cause photon migration from the surface of the head through the skin, skull, and underlying brain tissues to a detector 4 cm distant, also on the surface of the head. Absorption and/or scattering changes of light along the pathway of photon migration are sensitively detected.

The linkage between cerebral activity, oxidative metabolism, and oxygen delivery is demonstrated in animal models (2). The coupling between brain activity, metabolism, and blood flow is demonstrated in human subjects by positron-emission tomography (PET) scanning (3). Blood concentration increases are linked to optical measures of visual responses in the exposed primate brain (4); ^{1}H NMR shows localized blood flow changes on photic stimulation (5).

This study is methodologically similar to the optical spectroscopy of the exposed brain in animals (4). In this case, transcranial signals are obtained and human studies are possible by a simple dual wavelength spectrophotometer used to study the frontal region of the forehead and to measure changes of light absorption related to cerebral function (L.L., unpublished data). The instrument is responsive to changes of both blood concentration and deoxygenation/oxygenation changes that are associated with activated brain function (6) as measured in the frequency domain as recurrences in the 0.1- to 3-Hz region and bridges the gap between the relatively slow isotopic methods (spectrophotometry and PET) and faster responses of studies of electrical, magnetic, and electromagnetic field activity of the brain [electroencephalography (EEG), magnetoencephalography (MEG), and magnetic resonance imaging (MRI)]. This study seeks to answer the following question: are there low-frequency components of the blood concentration signal that are specific for functional activity of the human brain?

The possibility that the optical signals could be detected in the brain through the skin and skull is based on computer simulations and experimental studies (1, 7) of photon diffusion in scattering material of properties similar to brain. A crescent-like shape of the photon diffusion pattern is verified by pin hole scans of models with scattering properties similar to that of skin, skull, and brain, and the mean penetration depth of the migration is ≈2 cm—half the input/output separation of 4 cm. Photon migration also occurs in the brain tissues in the studies of Delpy *et al.* (8) and McCormick *et al.* (9), who used multiwavelength spectrophotometers to measure cerebral deoxygenation in humans without quantitation of the pathlength of photon migration; such techniques are limited to identifying trends of oxygenation due to the variability of the pathlength from subject to subject and, in a particular subject, the variation of the pathlength with changes in the concentration of the absorber. These effects confound the quantification of the continuous light method but do not invalidate its use as an indicator of changes of absorption/scattering as used here.

This paper records repetitive fluctuations in the optical signal in a 10-min stimulus interval. Thus, this method requires not only a blood concentration increase during stimulation but also periodical repetition of the increase within the tissue volume optically sampled. Such increases need not be repeated at identical locations but may have a spatiotemporal distribution. A 4-cm separation of input/output gives a mean penetration of 2 cm and a tissue volume of ≈5 ml within which the repetitive response is observed.

Experimental Method

The brain spectrophotometer (Fig. 1) employs two wavelengths of light. The sum of the absorbance change is appropriate to the detection of changes in the total blood concentration signal. The difference of the absorbancy changes gives changes in the balance of oxygenation/deoxygenation of hemoglobin in the red region of the spectrum—i.e., λ_1 = 760 nm and λ_2 = 850 nm. The changes in blood concentration are insensitive to the oxygenation/deoxygenation of hemoglobin (HbO_2/Hb) because approximately one-half of the 760-nm signal is added to the 850-nm signal and vice versa. The data reported here have been obtained in the sum mode, and the

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Abbreviations: PET, positron-emission tomography; EEG, electroencephalography; MEG, magnetoencephalography; MRI, magnetic resonance imaging.

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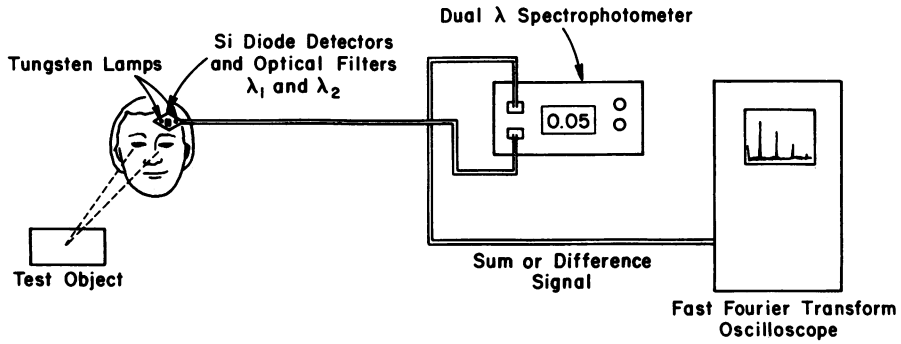

FIG. 1. Frequency analysis of test − rest signals in human brain. Diagram of low-frequency tissue spectrometer and Fourier transformer.

time dependence of changes of blood concentration have been measured. The system block diagram is shown in Fig. 1.

The optical probe consists of two tungsten flashlight bulbs, each placed 4 cm from the two silicon diodes (4 × 10 mm) and each equipped with an interference filter transmitting a 10-nm-wide band centered at 760 and 850 nm. Thus, this system provides two photon migration paths with input/output separation of 4 cm. A preamplifier couples the optical signals to an amplifier unit that takes the sum and difference of the signals suitably corrected as described above. The tungsten lamps are pulsed at 3 Hz so that absorbance measurements are time shared with background light to provide a correction via sample and hold techniques.

The output signal is directly connected to the fast Fourier transformer type 440 (Nicolet), which is used in the dc-coupled mode on the 200-μV scale. The conditions are set for 16 iterations in the 0- to 5-Hz scale for a total interval of data acquisition of 10 min. The recording sensitivity is 220 μV/cm at ×512 gain (Fig. 2*A*) and 440 μV/cm at ×256 gain. The noise level of Figs. 3–5 is ≈20 mV. The total signal is 115 mV and the electrical noise level is 0.02%. The full-scale sensitivity is 0.1% of absorbance change at ×512 and 0.2% at ×256.

The output of the sum circuit is filtered and the response reaches 70% in 1 sec, as shown in Fig. 2*B*. The square wave

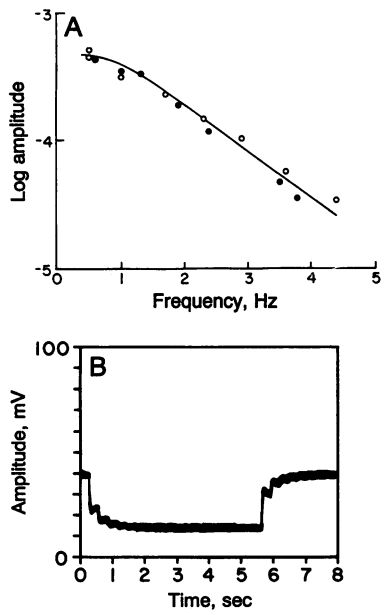

FIG. 2. (*A*) Illustration of the frequency response of the overall system to low-intensity light signals modulated at low frequencies. (*B*) Response speed test illustrating the response of the dc amplifier to a square wave of 5.5 sec duration. A 70% response is obtained in 1 sec.

optical signal is sustained in the boxcar integration of the detector circuit and is filtered thereafter to ensure that no "windowing effect" gives Fourier transform signals related to the light chopping.

The frequency response of the filtered output of the complete unit to a sinusoidal light input has been tested with a diode laser modulated with low-frequency sinusoids in the range 0.5–4 Hz. The attenuation characteristics shown in Fig. 2*B* are nearly flat to 1 Hz and are down 1 decade at 2–3 Hz. Thus, the signals above 1 Hz are relatively larger than shown, particularly in Fig. 6*B*. Direct coupled measurements carried out simultaneously with the Fourier transform study indicate that the increase of blood concentration is ≈2% of the total optical signal (L.L., unpublished data).

Subject Population. All except two of the subjects were participants in a minority student research training program and were juniors or seniors from Central and Girls High Schools in Philadelphia. Consent forms were signed by parents of the students and all studies were conducted under a University of Pennsylvania IRB-approved protocol. The subject was attended by an observer who noted attentiveness during test and sleep during the rest period. Head motion and mastication were minimized.

Controls. The ideal control would repeat every feature of the study except the specific changes due to recognition of the analogies. Thus, we repeated the study with two intervals of rest of duration equal to that of the rest − test study. The test − test is inappropriate because of the double duration of the test, which might lead to accommodation in the responses, and because the response to different tests may be different. Mapping of the test response by locating the probe in different parts of the head is feasible and has been used with localized hematoma as in adult head injury (6). (See *Possibility of an Artifactual Response of Subject* for a discussion of artifacts.)

Signals. Each recurrent signal is indicated by a peak at a particular frequency (Hz) in the illustrations. Some are present in the rest recording, usually arterial pulse and related frequencies. Additional recurrent frequencies are usually observed in the test interval, and the Fourier transforms are readily subtracted by the instrument (test − rest).

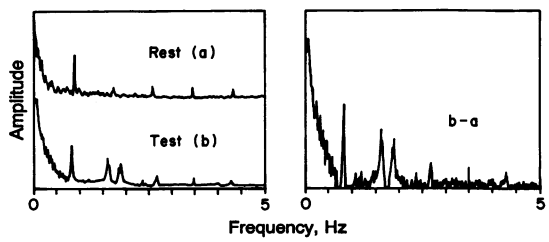

FIG. 3. Frequency analysis of optical signals obtained as described in Fig. 1. Subject U test (trace b), rest (trace a), and test − rest (trace b − trace a) Fourier transforms (n = 16; gain, ×256).

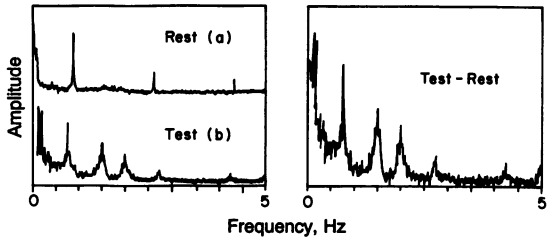

FIG. 4. Subject B test (trace b), rest (trace a), and test − rest Fourier transforms (n = 7; gain, ×512).

Nonrecurrent frequencies may also contribute to the spectra, especially in the low-frequency region, and increase as the frequency decreases, termed $1/f$ noise, and usually extends to 1–2 Hz but is observed to 5 Hz and has been observed to increase in the test interval (see below).

Relation to Arterial Pulse. At rest, a low-frequency component (≈1 Hz) is detected at various locations on the forehead and tracks closely the frequency of arterial pulse as detected on the wrist. However, these signals are nearly completely canceled in the test − rest difference Fourier and are thus attributed to the arterial pulse in the brain tissue. Occasionally the test − rest data show an increased arterial pulse rate during the test period.

Experimental Protocol

The Test. The subject was exposed to visual stimulation by a series of abstractions composed of analogies taken from SAT (Scholastic Aptitude Test) examinations. This was an appropriate challenge for the age group. Sixty of these abstractions were displayed for an intended time of 11 min required for 16 iterations of the Fourier transformer. The advance of one analogy to the next was dictated by the subject's conclusion that the analogy had been understood. Analogies were continued within the 11-min interval as long as the subject felt that he/she was adequately able to concentrate on them. If their attention was diminished due to fatigue, etc., the test was terminated at that point (see Fig. 4). Inattention during a test was not scored and no tests were rejected or selected. Usually >11 of the 16 iterations of the Fourier transform were obtained and often the full 16 were obtained. At this point, the subjects were told to rest as best they were able (no mastication was permitted in test or rest).

No analogies were presented during the rest intervals. The Fourier transforms of the optical data for two successive 11-min intervals were recorded and subtracted and are entitled rest − rest (Fig. 5).

Other Studies. These studies comprise a total of 31 tests of which 28 were presented abstractions and 24 showed recurrent frequencies during the test interval. Another study under separate authorship involved 7 tests of verbal analogy questions, with 30 questions per test (L.L., unpublished data). The first 3 tests were composed of questions taken at random from previous SAT examinations (set 1). The remaining 4 tests consisted of questions that were specially constructed to prevent imagery (set 2). Each subject responded to both sets

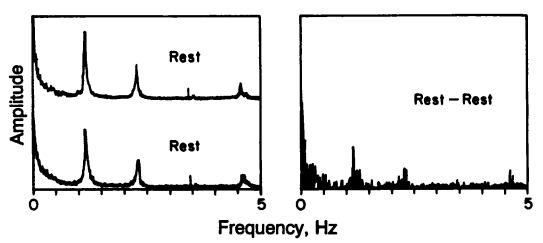

FIG. 5. Subject U rest − rest control study (n = 16; gain, ×256).

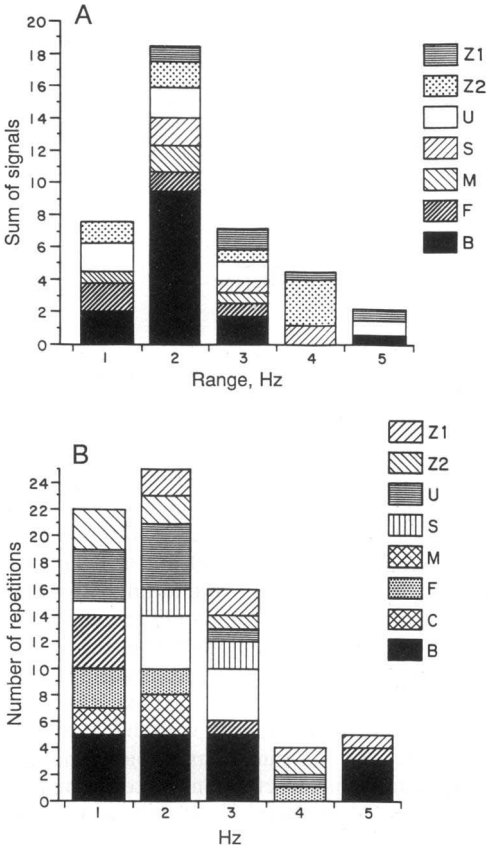

FIG. 6. (*A*) Histogram of distribution of frequencies observed in 28 test − rest studies involving 7 individuals. (*B*) Histogram display of distribution of energy (area under peaks) of the observed 28 test − rest studies of 8 individuals.

of analogy questions (one group per day). A 3rd test using noun–verb algorithms was used in 66 tests (unpublished data). The total test − rest time is estimated to be several hundred hours with three dozen subjects.

Results

Test − Rest. The Fourier transforms during the test interval and the rest interval are displayed as traces b and a, respectively, in Figs. 3 and 4. A test of a young female subject is shown in Fig. 3, where the frequency scale is 0–5 Hz, the amplitude scale represents a gain of ×256, and 16 of the 16 scans were obtained. In the rest spectrum, the predominant peak is at 0.8 Hz, ≈2% of the total signal (Fig. 2*A*). Small, sharp peaks appear at 1.7, 2.7, 3.5, and 4.7 Hz. Similar peaks of similar amplitude appear in the test study and are thus not activity related. In the test spectrum, large peaks appear at 1.6 and 1.8 Hz.

The difference of the two Fourier transforms designated test − rest (trace b − trace a) shows a recurrence of signals at particular frequencies associated with presentation of the analogies. There are peaks at 0.8, 1.6, and 1.8 Hz and a small peak at 2.8 Hz. The 0.8-Hz peak appears in both rest and test and corresponds to 1% of the total signal. This is attributed to the arterial pulse. The 1.6-, 1.8-, and 2.8-Hz peaks appear to be activity related. Of interest is a large component of low-frequency noise in the test − rest diagram. The doublet peaks at 1.7 and 1.9 Hz recur in repeated tests.

A test of another young female (subject B) is shown in Fig. 4, where the rest spectrum shows a strong peak at 0.8 Hz in both rest and test due to the arterial pulse and a second peak at 2.7 Hz. After 7 of the usual 16 iterations, the subject spontaneously ceased the study. The rest interval was sim-

ilarly curtailed. The Fourier transform of the test − rest spectrum contains broad peaks at 0.8, 1.5, 2.0, 2.7, and 4.3 Hz. The largest peak exceeds 1% of the total signal. The low-frequency noise is greater in test than rest and extends to 2 Hz.

Rest − Rest. Very small differences are obtained if the subject is at rest in both intervals. As shown in Fig. 5, the two rest spectra show somewhat stronger peaks than were found in the rest study of Fig. 3 at 1.2 and 2.3 Hz, with a small peak at 4.6 Hz. However, the area under the peaks is much less than the activity-related signals of Fig. 4.

Summary of All Tests

Histograms. The histogram of Fig. 6 displays the recurrence of frequencies in the range 0.5–1.5 Hz, 1.5–2.5 Hz, etc. A portion of the 1-Hz signals is usually due to an arterial pulse increment. The occurrences and energies shown in Fig. 6 are diminished by the frequency response illustrated in Fig. 2.

The area under the trace at a particular frequency gives the power spectrum shown in Fig. 6*B* for 1-Hz intervals. The largest area is at 1.5–2.5 Hz.

Discussion

Origin of the Optical Signal: Relation to the Steady Component. The recurrent fluctuations observed here are superimposed on a steady component of blood concentration change during activity. A mean 2% absorbance decrease during activity with respect to rest is found in 43 studies of six subjects by the same protocol (L.L., unpublished data). Thus, the fluctuations observed by Fourier transform are a small fraction of the steady component (i.e., 10%).

The Blood Signal. The isosbestic point in the Hb/HbO_2 spectrum at 800 nm and the balanced sum of Hb/HbO_2 absorbances at 760 and 850 nm give similar extinction coefficient/concentration products of hemoglobin that represents the principal absorber at these wavelengths. The Hb absorbancies are >20 times that of cytochrome aa_3. Water is also a smaller absorber at these wavelengths. The differences of absorption at the two wavelengths are frequently recorded but showed small signals, and thus oxygenation/deoxygenation is not the predominant effect in these studies. Changes of light scattering are not separated from absorbance changes when using continuous light and would be expected to uniformly affect both 760 and 850 nm and would be detected as a blood volume change. Light-scattering changes are known to accompany electrical activity of neurons (10, 11) and may be present in these studies. However, other methods—PET, spectrophotometry, and MRI—show the existence of blood flow changes (3, 5). Since this instrument sensitively responds to blood concentration changes, results are consistent with the blood volume changes measured by the other methods.

These correlations, together with the sensitive response to the arterial pulse observation, lead us to conclude that the rest − test signals are due to blood concentration changes and the fact that the arterial pulse is the strongest signal observed equally in both rest and test is strongly supportive of this identification.

The Repetitive Response. One explanation of the low-frequency oscillation of blood concentration is based on well identified metabolic oscillations (12). Spatiotemporal variations are observed in studies of the heterogeneous response of the fluorescence of brain NADH (13) and of the myocardium in low-flow ischemia (14). Spatiotemporal heterogeneity and recurrent patterns of altered metabolism are observed in both processes. In brain, the kinetic patterns of receptor binding may be a part of a fundamental cognition process with particular recurrence frequencies.

Comparison with Other Methods. There are basic differences in the optical and PET measurements. PET gives an integrated response over 40 sec to the increased isotope concentration in the blood vessels of stimulated neurons as compared with nearby neurons that were not stimulated. PET further averages repeated responses from several individuals (3). Grinvald and coworkers (4) similarly integrate the optical signal of an iterated stimulus.

The results may also be compared with similarly fast methods such as MEG or EEG since both methods employ Fourier transform frequency analysis. However, the frequency ranges are not overlapping, generally because of the larger $1/f$ noise of both MEG and EEG as compared with the optical method rendering these low frequencies 0.5–3 Hz less accessible, thus the 8 Hz and higher region.

Recent developments in the speed and sensitivity of MRI show localized responses to visual and sensory motor stimulation and to auditory stimulation in the respective occipital, parietal, and temporal regions (5). The last study shows on/off responses to music consistent with a greater Hb concentration. The increase of Hb requires several seconds as observed in the occipital cortex, but no recurrence frequencies are observed.

Reproducibility of the Frequency Spectrum. The preliminary results indicate a slight variation of the pattern of frequencies recorded for a given individual in successive tests. To avoid accommodation to the test, different sets of abstractions were presented and thus different responses were expected. However, a few individuals in a given day reproduced two of the three frequencies in successive tests.

Nonrepetitive Responses. The responses that lack coherence will be stored as $1/f$ noise as shown in Fig. 3 as is characteristic of some responders. In these cases, activation of increased blood concentration changes within the optical field are observed but do not show a repeat pattern that is distinguishable in the 10-sec recording interval.

Other Responses. The students tested were appropriately challenged by the questions used in these cognition studies. With other age groups (to be reported elsewhere), a small portion of the subjects show both no response (no change rest − test) or a negative response (rest − test signals disappear in test).

Bandwidth. Our narrow band width (0–3 Hz) and relatively short iteration time (<11 min) in this study may show only a portion of the total frequency spectrum. It is possible to construct a much faster instrument.

However, there are limits to the response speed of blood volume and metabolic responses. NMR studies indicate that blood concentration changes occur within a few seconds in the response of the visual cortex of the cat. Our data suggest that the repetitive fluctuations in the human brain extend up to 2–3 Hz.

Analogies. The analogies presented to the subjects in this study were intended to stimulate associative responses similar to the noun–verb association used by Raichle and coworkers (3). In his study, a PET image of increased blood flow was calculated from the average of data from the 5 subjects to give a clear response of increased flow in the left prefrontal cortex of the subjects. It appears reasonable to assume that the associative abstractions we used give a similarly localized response.

Localization. Photon migration patterns in brain tissues are modeled experimentally with lipid scatterers with skin, skull, and brain tissue models and with Monte Carlo and diffusion equation simulations (7). The penetration of skin and skull and migration through brain is verified for the input/output geometry used. The fraction of the signal attributed to the skin surface layers is very small since optical pathlengths in this region are very short because of the high probability of proton escape, and only the deeper layers afford longer

pathlengths that have a higher probability of transit from input to output. The optical system was designed to respond optimally to blood concentration changes, a volume of 3–5 ml, in the frontal/temporal cortex, and the problem set was designed to stimulate the frontal region. When left and right sides of the forehead were compared, the success rate of new peaks in the Fourier transform was observed to be 2 times greater on the left side (B.C., unpublished observation).

Possibility of an Artifactual Response of Subject. We have used a number of tests to identify artifacts. In each study, a rest and test Fourier transform was observed, and only those peaks appearing in the test have been listed. The possibility that blood volume changes within the optical field accompanying the mentation are caused by some other phenomena—for example, mechanical movement such as mastication, wrinkling of brow, etc.—during mentation was examined. These movements were excluded by observations of the subject in the test and rest periods but would not be expected to show a high-recurrence frequency. Sleep during the rest period was minimized by the observer.

Future Possibilities

The most important aspect of future development of the optical methods is the rate at which data may be accumulated. The travel time of photon migration over a pathlength of 1 m is 23 nsec, with a 4-cm input/output separation. The photon migration pattern should be essentially complete in this distance, allowing a repetition rate of at least 50 MHz. This compares very favorably with the time limitations of PET and MRI. The signal/noise ratio will determine the number of iterations of the test, which in these preliminary studies was ≈50 over a period of 11 min. However, very primitive localization was used. To this point, time slicing of the time domain study with optimized input/output positions measured simultaneously on the forehead would be expected to reduce the integration time by a factor of 10. Either pulse time or phase modulation technology seems appropriate to such a study.

The photon migration kinetics are responsive to both light scattering and light absorption and the two parameters may be resolved by time slicing or frequency variation. While absorption has been stressed in these studies, light scattering is much closer to the primary events of neuronal/axonal response than the blood flow/blood concentration change both in anatomy and in time. Thus, future studies may use localized light-scattering changes with response capabilities in the kilohertz region and short averaging times.

The objective evaluation of functional activity of the brain with a simple and inexpensive device would open up a wide range of studies of performance in responding to appropriate tasks that are currently restricted to very expensive techniques (MEG and EEG) or to techniques that involve the use of radioactive isotopes. Thus, screening for deterioration of brain function might be studied conveniently and continuously.

Summary

The possibility that a simple device, a dual wavelength direct-coupled spectrophotometer, can detect changes in the low-frequency components of the light absorption signal from the human brain in response to brain function activity involving abstract thinking tests deserves thoughtful consideration and further experimentation. In this study, the low-frequency recurrence of changes of blood concentration in portions of the brain that lie ≈2 cm deep from the surface of the skull of the subjects was examined. The application to other stimuli, which affect function in other regions of the brain, may be possible. It is appropriate to speculate that the recurrence frequencies observed in the cognitive tests would be significantly diminished in cases of neuronal deterioration. Thus, a simple noninvasive and rapid test for cognitive function of the frontal region of the brain seems to be available.

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