strates. The higher activities obtained with the nonradioactive substrates are probably due to the higher concentrations of substrates used (about twice the Michaelis constant values found for the crude enzymes) or to a higher enzyme affinity for the nonreduced substrates in the pyranose ring conformation.

No inhibition of normal sulfatase activities was detected when extracts of normal fibroblasts, mixed in various proportions with extracts of fibroblasts from Morquio patients or from patient G.G., were incubated with the various substrates. With all substrates used, the enzymatic activities of normal fibroblast extracts were linear with respect to increases in time of incubation or protein concentration. The *p*H optimum for the sulfatase activities measured with the various substrates was 4.8, in agreement with the value found previously with chondroitin-6-sulfate tetrasaccharides (*5*). Half-maximal activities were observed at *p*H 3.7 and 5.6. Despite these similarities between the two postulated 6-sulfatases, their different sensitivities to lyophilization, concentration of ammonium sulfate or various ions, and distribution in various organs (*14*) strongly suggest that they represent different proteins.

These findings, obtained either with nonradioactive substrates (which occur in keratan sulfate, heparan sulfate, and chondroitin-6-sulfate) or with more convenient, radioactive derivatives of the substrates, support the suggestion (*3–5*) that the deficient enzyme in classical Morquio disease is a 6-sulfatase specific for substrates with the galactose configuration (*N*-acetylgalactosamine-6-sulfate and galactose-6-sulfate). Moreover, they demonstrate the existence of a separate 6-sulfatase, specific for substrates with the glucose configuration (*N*-acetylglucosamine-6-sulfate), whose deficiency is responsible for a novel mucopolysaccharidosis which affects the degradation of keratan sulfate and heparan sulfate and has clinical and biochemical features which may be considered a composite of those of Morquio and Sanfilippo phenotypes.

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Somatically Evoked Magnetic Fields of the Human Brain

Abstract. *The human brain is found to produce a magnetic field near the scalp which varies in synchrony with periodic electrical stimulation applied to a finger. Use of a highly sensitive superconducting quantum interference device as a magnetic field detector reveals that the brain's field is sharply localized over the primary projection area of the sensory cortex contralateral to the digit being stimulated. The phase of the response at the stimulus frequency varies monotonically with the repetition rate and at intermediate frequencies yields a latency of approximately 70 milliseconds for cortical response.*

We report here the detection of magnetic fields associated with the flow of electric current in the brain in response to electrical stimulation of the fingers. Weak magnetic fields resulting from visual stimulation have previously been detected outside the scalp (*1–4*). In contrast to the diffuse nature of the visually evoked potential (VEP), which is conventionally measured with scalp electrodes, the visually evoked field (VEF) is located over the visual cortex (*2*). This is to be expected as the electric currents giving rise to the VEF flow within the visual projection areas of the brain, while accompanying weaker currents in the skin remain undetected. The VEF has proved to be a significant indicator of brain function since its latency is directly correlated with the reaction time of a subject when the spatial frequency of a stimulus is varied (*5*, *6*). The confined location of the VEF and its correlation with a measure of human performance prompted us to search for neuromagnetic responses evoked by other stimuli. The neuromagnetic response evoked by electrical stimulation of individual fingers of the hand—the somatically evoked field (SEF)—is similarly found to be well localized over the primary projection area, in this case on the contralateral hemisphere in the region SI for the stimulated digit. The observed features of the SEF indicate that the neuromagnetic techniques can provide unique advantages for medical research.

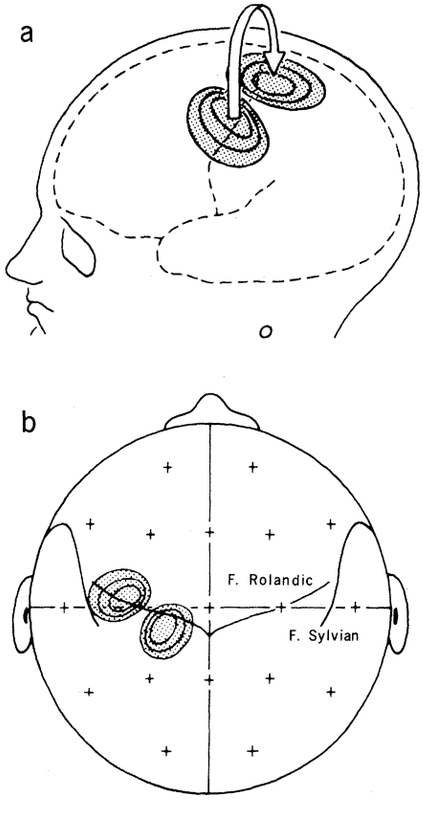

Fig. 1. (a) Neuromagnetic field patterns on the left hemisphere for an electrical stimulus at 13 hertz applied to the little finger of the right hand. Contours of equal magnetic flux indicate the relative amplitude of response for 0.9, 0.7, and 0.5 of the maximum response at the stimulus frequency. (b) The same pattern drawn on the conventional 10–20 electrode map. Abbreviation: *F.*, fissure.

A split-ring electrode, fashioned from a rubber grommet and two stainless steel

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tabs, was used to apply 1-msec d-c pulses (1 ma) transcutaneously to either the little finger or the thumb at frequencies ranging from 3 to 30 pulses per second. Stimuli at all frequencies were distinctly experienced and described as moderate by all subjects. The evoked magnetic field was detected near the scalp by a superconducting quantum interference device (SQUID), using a flux transporter wound in the configuration of a second-derivative gradiometer, which is insensitive to uniform background fields and to background fields with uniform spatial gradients. An earlier version of this detector has been described elsewhere (*7*), and the principles of its operation have been explained (*6*). The lowest or "pickup" coil of the gradiometer was positioned 7 mm from and tangential to the scalp in order to detect the component of the field normal to the scalp. The coil diameter was 2.4 cm and the gradiometer baseline between adjacent coils was 3.2 cm. Both the SQUID and the gradiometer were immersed in liquid helium contained in a fiber glass Dewar positioned near the head. The SQUID electronics (*8*) provide an output voltage proportional to the magnetic flux linking the pickup coil. Like all of our previous experiments, these studies were carried out in a normal laboratory environment without magnetic shielding.

The detector output was amplified, band-pass filtered at the stimulus frequency, and averaged in real time over each 1-minute trial by a Waveform Eductor (*9*). The averaged SEF component at the stimulus frequency exhibited an amplitude ranging from 0.1 to 0.5 picotesla (1 tesla = 10^4 gauss), depending on frequency. Despite environmental background fields of approximately 5 microteslas the noise rejection of our system provided a signal-to-noise ratio which ranged from 4 to 15 for the averaged signal. Experiments were performed with the subjects prone on an inclined plane, with their heads turned completely to one side or the other (*10*).

The field pattern for the most extensively studied subject is illustrated in Fig. 1. The magnetic field linking the pickup coil during the illustrated half of the response cycle emerges from one small region of the scalp and enters another close by. During the next half-cycle the field direction is reversed. The diameter of each of the two regions where the detected flux exceeds half of its maximum value is about 4 cm. This is an overestimate of the true size of the field pattern because of the limited resolution imposed by the relatively large diameter of our pickup coil. The area between the centers of the two regions appears to lie directly over the primary sensory projection area for the contralateral little finger. This pattern is qualitatively similar to that which would be associated with a current dipole source (*11, 12*) located just under the scalp midway between the two regions and oriented parallel to the scalp but perpendicular to a line passing through the centers of the two regions.

The field pattern is reversed for the contralateral projection area when the little finger of the other hand is stimulated, indicating that patterns of current flow have bilateral symmetry. This result is not unexpected. When the thumb is stimulated instead, the entire pattern shifts downward by approximately 2 cm. This correspondence with the ordering of the somatic projection areas for the various digits (*13*) provides additional evidence for the cortical origin of the neuromagnetic response.

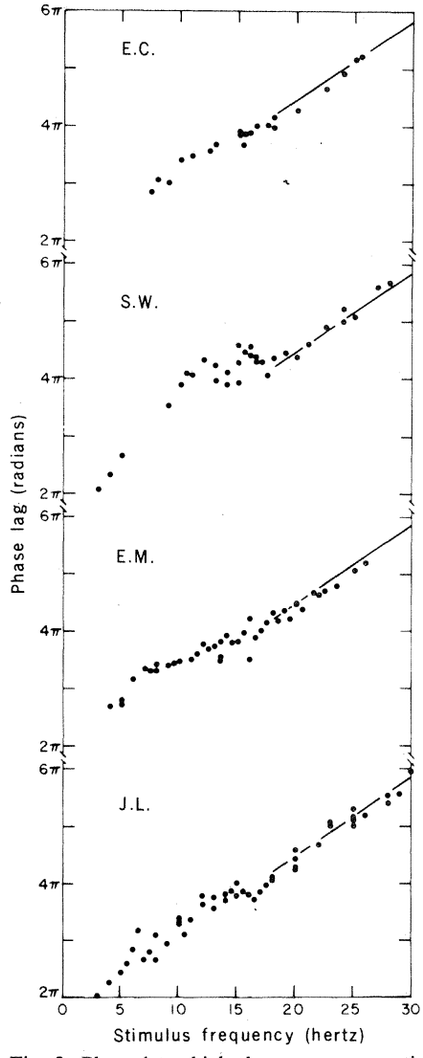

Fig. 2. Phase by which the average somatically evoked field from each of four subjects lags behind the stimulus for various stimulus frequencies. The solid line above 18 hertz is the best-fit line for data from all subjects. The phase values begin at 2π to ensure that the extrapolated values of phase lag for frequencies below 3 hertz are positive.

In addition to the field patterns, we studied the phase of the average response at the stimulus frequency over the range 3 to 30 hertz. We arbitrarily define phase as the occurrence of the maximum outward field in the upper region of the left hemisphere (Fig. 1) relative to the onset of the stimulus. For our moderate levels of stimulation, the phase of the response at a particular frequency was found to be independent of the stimulus amplitude for each of the subjects. Phase was also found to vary monotonically with frequency, as shown in Fig. 2, with all four subjects exhibiting a common linear trend above 18 hertz. Below 18 hertz the data display considerably more intersubject variability. A trend analysis within the context of an analysis of variance was performed for all data pooled together. Data were censored across subjects to ensure that at least two observations per frequency for each subject were included. The F ratio for linear regression is significant at $P > .01$. The linear variation of phase with frequency can be characterized by a response latency of 61 ± 5 msec, as derived from the slope (*14*). Including only data above 18 hertz, where intersubject variability is considerably reduced, yields a latency of 70 ± 7 msec, with individual latencies ranging from 62 ± 8 to 79 ± 5 msec.

In separate psychophysical studies, the mean of the reaction times for one finger depressing a button in response to a single pulse to the little finger of the other hand was found to be 172 ± 11 msec for the same four subjects. The difference of 102 msec between this and the neuromagnetic latency for responses above 18 hertz (or 111 msec for all the pooled data) can be interpreted as the motor response time. We note that this value is comparable to the 115-msec value for the motor response time previously deduced from reaction times and neuromagnetic latency measurements with visual stimulation (*5*).

The origin of the larger phase variance between subjects when the stimulus frequency was less than 18 hertz is not understood at present. Subjects E.C., S.W., and J.L. yielded responses in a broad region around 15 hertz that seemed to be independent of stimulus frequency. This phenomenon is not evident in the data for E.M. Even so, the nonlinearity of the phase trends suggests

that several different fiber tracts may be differentially activated by stimuli as a function of frequency. This problem is suitable for further study.

The results reported here demonstrate that the SEF may provide information about brain activity that differs significantly from what can be observed with other methods. Most striking is the sharp localization of the field over the active region of the cortex, in contrast to the diffuse nature of potential recordings. Components of the transient SEP with a latency of 40 msec or greater are found over regions of both hemispheres (*15*), as opposed to the contralateral characteristic of the steady-state SEF. The generally good reproducibility of the phase of the SEF also makes neuromagnetic measurements attractive as a means of determining sensory response time, which should have clinical applications where estimates of nerve conduction velocity are desired. The opportunities for noninvasive mapping in the determination of sensory deficits as well as the study of sensory interactions all provide subjects for future investigation.

We conclude that for periodic somatic and visual stimulation, the neuromagnetic latency is a measure of neural transmission, with motor response times approximately constant regardless of stimulus modality.

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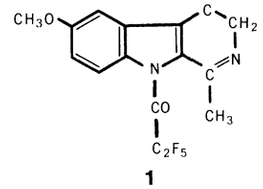
Melatonin Content of the Human Pineal Gland

Abstract. *Gas chromatography with electron capture detection was used to quantitate melatonin in single human pineal glands. The sensitivity of this melatonin assay is in the low picogram range. A 24-hour rhythm of pineal melatonin content was observed.*

Melatonin is a pineal hormone that acts on the gonads of rats (*1*). It modifies sleep, electroencephalographic activity, and serotonin content of the brain (*2*). It has also been implicated in the pathogenesis of schizophrenia (*3*). In mammals, the enzyme hydroxyindole-*O*-methyltransferase (E.C. 2.6.1.27), which is responsible for the biosynthesis of melatonin from serotonin, is found mainly in the mammalian pineal gland, and this gland is probably its only site of production (*4*).

Using the technique of gas chromatography with electron capture detection, we have devised two procedures for the quantitative analysis of pineal melatonin content. In both procedures volatile derivatives are formed by reacting the hormone with fluorinated acid anhydrides. These derivatives are fluorinated analogs of β-carboline. Initially, we used the anhydride trifluoroacetic anhydride (TFAA); later we found that pentafluoropropionic anhydride (PFPA) produced a more sensitive derivative and was the reagent of choice.

The structure of the pentafluoropropionyl (PFP) derivative of melatonin (**1**) was given by Cattabeni *et al.* (*5*). Its molecular weight is 360. Using a Micromass 12 gas chromatograph–mass spectrometer we obtained a mass spectrum of the trifluoroacetyl (TFA) derivative of melatonin, with major ions at mass-to-charge ratios (*m/e*) of 310, 213, 198, 186, and 170. This could be interpreted in terms of a structure similar to (**1**), with the TFA group replacing the PFP group. The molecular weight of the TFA derivative is 310.



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The pineal glands were separately homogenized at 0°C with 1 ml of phosphate buffer, *p*H 7, by means of a ground-glass tissue grinder. Another 1 ml of the buffer was used for washing the minced tissue. The homogenate was then shaken with 5 ml of petroleum ether and centrifuged. The aqueous layer was removed and saturated with anhydrous ammonium carbonate powder and extracted with 5 ml of chloroform. The organic layer was then washed successively with 5 ml each of 0.05*N* NaOH and 0.1*N* HCl. The chloroform solution was then dried under a stream of nitrogen. The residue was reconstituted with the appropriate solvent for derivatization. The recovery of melatonin by this method is estimated to be 70 percent.

The first set of results was obtained by TFA derivative formation. Here the residue was reconstituted with 100 μl of ethyl acetate and reacted with 200 μl of TFAA for 30 minutes at 80°C. The reaction mixture was then dried and reconstituted with 100 μl of ethyl acetate; this solution (2 μl) was injected into the gas chromatograph for analysis. For quantitation, solutions of authentic melatonin were prepared, and their TFA derivatives formed. Calculations were based on peak heights. The minimum measurable concentration of melatonin with this assay is about 3 ng per gland.

In later experiments we prepared the PFP derivative of melatonin by reacting the hormone with PFPA in the presence of a basic catalyst. This derivative gives a much better response to electron capture detection. The second set of data was generated with this assay. Here the residue of the pineal extract was reconstituted with 100 μl of 1 percent solution of triethylamine in benzene and reacted with 50 μl of PFPA at 70°C for 20

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