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.

D. BRENNER
Departments of Physics and *Psychology, New York University, New York 10003*
J. LIPTON
L. KAUFMAN
Department of Psychology, New York University
S. J. WILLIAMSON
Department of Physics, New York University

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16. Supported by the New York State Health Research Council (contract C106219), the Office of Naval Research (contract 00014-76-C-0568), and the National Science Foundation (grant BNS75-13162). We thank E. E. Coons for informative and stimulating discussions and E. Maclin for technical assistance.

19 May 1977; revised 17 August 1977

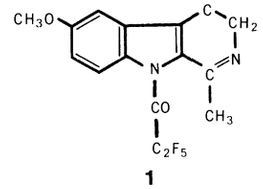
Melatonin Content of the Human Pineal Gland

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.



1

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

0036-8075/78/0106-0083$01.00/0

minutes. The reaction mixture was then dried and reconstituted with 100 μl of a solution of dieldrin in benzene (75 mg/liter), and washed with 300 μl of water. This solution (2 μl) was injected into the gas chromatograph. The dieldrin was used as an internal standard. Solutions of authentic melatonin of appropriate strength were prepared for the construction of the calibration curve. The ratio of the peak height of the PFP derivative to that of dieldrin was used to calculate the melatonin content. The minimum concentration of melatonin that could be measured with this assay is about 0.5 ng per pineal. As little as 10 pg of melatonin (in the form of the PFP derivative) per injection could be detected, and linearity was maintained up to at least 800 pg.

We used a Hewlett-Packard 5713A gas chromotograph fitted with a ^{63}Ni electron capture detector. The silanized glass column 2 m by 3 mm (inner diameter) was packed with 3 percent OV-225 on Chromosorb W (HP), 80/100 mesh. The carrier gas was 5 percent methane in argon (flow rate, 30 ml/min; column temperature, 230°C; injection port temperature, 250°C; detector temperature, 300°C). Under these conditions the retention times of the TFA and PFP derivatives and of dieldrin are 5.0, 3.8, and 4.2 minutes, respectively. Identification of the melatonin derivative was based on retention time. In addition, in the case of TFA derivative of melatonin, confirmation was carried out on a different column [either 3 percent OV-17 or 3 percent SE-30 on Chromosorb W (HP)]; for the PFP derivative, the pineal extract was reanalyzed with a Finnigan 4000 gas chromatograph–mass spectrometer according to the technique of mass fragmentography, and the parent ion (*m/e*, 360) was monitored. A typical chromatogram of the pineal extract by PFPA derivatization is presented in Fig. 1.

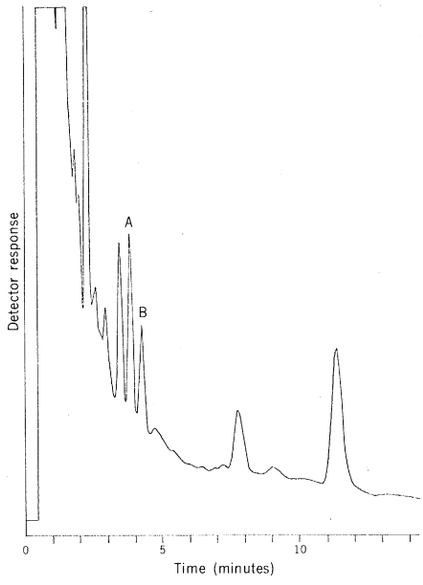


Fig. 1. A typical gas chromatogram of the pineal extract by PFPA derivatization. The two peaks of interests are (A) the PFP derivative of melatonin, with 200 pg of melatonin, and (B) dieldrin, with 150 pg of melatonin.

We analyzed the pineal glands of 22 nonschizophrenic patients. Table 1 shows two sets of data. The first set, indicated by asterisks, was collected in 1976, and the melatonin derivative was formed with TFAA. The second set of data was collected in 1977, and the derivative was formed with PFPA. A diurnal variation of the pineal melatonin content was observed. The melatonin concentration increased in the evening and through the night and started to fall in the morning, reaching a minimum around noon. This is in agreement with the observed 24-hour rhythm in the melatonin content of the pineal in rats, quails, and chickens (*6*). A similar pattern was also demonstrated in the melatonin content of human urine (*7*) and plasma (*8*). Wurtman *et al.* (*9*) found that the melatonin content of the metastasis from a human pinealoma was approximately 300 ng per gram of tissue. It is of interest that the concentration of melatonin in the human pineal gland is not much higher than that in rats [0.5 to 6.8 ng per pineal (*6*)], although the human pineal body is 100 times heavier. The 24-hour urinary outputs in rats (*10*) and in the human (*7*) are also of the same order of magnitude (1.16 ng and 8.9 ng per day, respectively). The concentration of pineal serotonin, the precursor of melatonin, also shows a similar pattern (*11*).

Table 1. Melatonin content of pineal glands of nonschizophrenic subjects.

Patient	Time of death		Age (years)	Sex	Melatonin (ng/gland)	Cause of death
	Hour	Month				
1*	0025	March	76	F	15.0	Pulmonary embolism
2*	0105	April	59	F	25.5	Myocardial infarction
3	0350	January	53	F	5.8	Strangulation
4	0510	April	59	M	71.1	Myocardial infarction, alcoholic liver disease
5*	0900	January	56	M	4.7	Cerebral hemorrhage
6	0915	February	60	M	10.0	Second-degree burns (50 to 60 percent of body)
7	0945	January	48	M	1.7	Accident
8	1004	January	71	F	0.6	Myocardial infarction
9*	1010	March	60	F	0	Myocardial infarction
10*	1020	March	82	M	0	Myocardial infarction
11*	1305	August	44	M	0	Myocardial infarction
12	1335	March	81	F	0	Sideroblastic anemia (idiopathic)
13*	1335	April	42	M	7.5	Cancer of genitourinary tract
14	1615	June	85	F	0	Myocardial infarction
15*	1730	March	64	M	5.0	Myocardial infarction
16*	1730	April	49	M	27.3	Diabetes mellitus
17*	1745	May	63	M	0	Shock
18*	1745	May	43	M	16.7	Diabetes mellitus
19	1845	January	57	M	8.9	Myocardial infarction
20	1945	February	70	M	1.4	Myocardial infarction
21	2307	January	53	M	1.0	Myocardial infarction
22	2330	August	76	F	5.7	Myocardial infarction

*Data collected in 1976. Other data were collected in 1977.

A. C. GREINER
North Lawn Unit, Riverview Hospital, Essondale, British Columbia, Canada VOM 1J0

S. C. CHAN
North Lawn Unit, Riverview Hospital, and *Department of Chemistry, University of British Columbia, Vancouver*

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12 September 1977