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# Piezoelectricity in the human pineal gland

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**Abstract**

Melatonin secretion by the pineal gland has been reported to be affected by exposure to electromagnetic fields (EMFs). In an initial investigation to determine if calcifications commonly found in the pineal gland could respond to EMFs by a transducer mechanism, studies were conducted to ascertain if pineal tissues were piezoelectric. Second harmonic generation (SHG) measurements showed that pineal tissues contained noncentrosymmetric crystals, thus proving the presence of piezoelectricity. Both mulberry-like and faceted crystalline calcifications were observed by scanning electron microscopy (SEM). Some of the calcifications had compositions similar to that of hydroxyapatite; others contained a high concentration of aluminum.

*Keywords:* Aluminum; Calcification; Crystals; Electromagnetic fields; Scanning electron microscopy (SEM); Second harmonic generation (SHG)

## 1. Introduction

There is evidence that melatonin secretion by the pineal gland is affected by exposure to electromagnetic fields (EMFs) [1], but the mechanism by which the EMF is converted into intracellular second messengers that regulate melatonin gene expression is unknown. The pineal contains unusual calcified deposits that are chemically similar to bone mineral [2], and it occurred to us that the presence of calcifications and the sensitivity of the pineal to EMFs might be related.

Pineal calcifications occur in subjects of any age [3], but apparently in amounts that are relatively independent of age [4]. Neither the mechanism of formation nor the physiological significance of pineal calcifications are known [5]. There is microscopic evidence of an intimate association between the calcifications and cellular membranes [6]. Pineal calcifications have been given numerous names in the literature, including corpora arenacea, acervuli, psammoma bodies and brain sand [6].

Piezoelectricity is a third-rank tensorial property exhibited by members of the 20 noncentrosymmetric crystal point groups [7]. (In addition, a 21st point group 432 is also noncentrosymmetric but its members are not piezoelectric because of the presence of other elements of crystallographic symmetry.) In the direct piezoelectric effect, an elastic stress gives rise to a voltage; in the converse effect, an applied voltage results in elastic strain. If the pineal calcifications were piezoelectric, they could produce a surface charge distribution and a strain by virtue of the interaction of the direct and the converse piezoelectric effects whenever a subject was exposed to an appropriate EMF. In principle, either the electrical or mechanical changes could trigger intracellular second messengers that regulate the metabolism of pinealocytes.

The principal objective of this research was to determine whether the calcifications present in the human pineal gland were piezoelectric. The classical methods for measuring piezoelectricity [8,9] are not suitable for examination of specimens containing small piezoelectric crystals dispersed in a nonpiezoelectric material. Consequently, an alternative technique that would detect noncentrosymmetry was selected: second harmonic generation (SHG) [10,11].

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A positive SHG response is proof of the presence of piezoelectric crystals.

## 2. Experimental methods

In the technique of SHG, a sufficiently intense light wave of frequency ω is focused on a crystal. If the crystal is noncentrosymmetric, the electric field of the light wave induces a polarization at twice the incident frequency causing the crystal to emit light at double the frequency or half the wavelength. Kurtz and Dougherty [11] have presented a statistical analysis for the sensitivity of SHG in determination of noncentrosymmetry in powders.

The SHG detection technique used in our studies was as follows. The beam from a pulsed neodymium YAG laser emitting 15 ns pulses of approximately 4 mJ energy at a wavelength of 1064 nm was focused to a diameter of about 500 μm on the sample. An absorption filter was used to remove the 1064 nm component from the radiation reflected from the sample. The remaining radiation passed through a monochromator and then was analyzed by a high gain photomultiplier with photon counting sensitivity. Incident photons were counted for 200 pulses of the laser. When the monochromator was set with a 532-nm window, both SHG and any spurious background signal (which may originate from instrument noise, luminescence, or thermally excited processes) would be measured. The background contribution was determined by measurement with the monochromator set at a wavelength either 15—20 nm above or below the SHG wavelength. Thus the ratio of the photon counts at 532 nm to that at nearby wavelengths formed a signal-to-noise ratio. In addition, signals proportional to the intensity of both the incident 1064-nm radiation and the detected radiation were displayed on a dual-beam oscilloscope. If the detected radiation was not coincident in time with the incident radiation (with resolution of approximately 10 ns), it was assumed that thermal processes, which were significantly slower than those due to SHG, were being observed. The functioning of the SHG system was checked prior to each set of experiments using a sample of powdered urea which gives a very large SHG signal (measured at about $3 \times 10^5$ photon counts per 200 laser pulses).

All SHG measurements were made on pineal glands from six human cadavers of both sexes, 45—78 years of age. In most instances, regions of the pituitary gland, the cortex and the cerebellum were also measured as controls. The tissues were fixed in absolute alcohol (except in buffered formalin, in one case), sliced using a scalpel, placed on glass slides with a cover slip, and air-dried under slight pressure. The resulting preparations were 100—300 μm thick. For atomic absorption determinations of aluminum, the tissues from four cadavers were frozen at —70°C until analyzed. To determine the thermal stability of pineal crystals, the entire pineal glands from five additional cadavers were fixed in buffered formalin and then ashed at 200°C for 2 h. The resulting material (about 50 mg) was analyzed by SHG, X-ray diffraction, and SEM.

Because the samples were opaque or only slightly translucent, SHG measurements were made in a reflection mode (at approximately 45° incidence). No detectable SHG was observed from the glass cover slides, as evidenced by blank measurements without a sample. In an experiment, the laser was focused at an arbitrary point on the surface of a sample with the monochromator window at one of the settings, 532 nm, above 532 nm (540—550) or below 532 nm (510—520). Sets of 200 laser pulses were produced several times for each window setting. Then the laser beam impingement point was moved to another arbitrary location.

After the SHG measurements, a conductive gold coating was evaporated on some of the samples and they were examined by a scanning electron microscope (SEM) using a 25 kV beam voltage. Crystals and crystal-like regions were studied. Energy dispersive X-ray spectroscopy (EDS) was used for a quantitative analysis of chemical elements with an atomic number of 11 or greater.

X-ray diffraction and atomic absorption studies were carried out using conventional techniques.

## 3. Results

The results of the SHG measurements on the tissues from Subject 1 are illustrated in Fig. 1, and the results on tissues from all of the subjects are shown in Fig. 2. The following criteria were used to determine if SHG was observed at a specific location in a sample: (1) the number of photon counts for 200 laser pulses was greater than 10 and, (2) the number of photon counts with the monochromator set at 532 nm was statistically significant compared

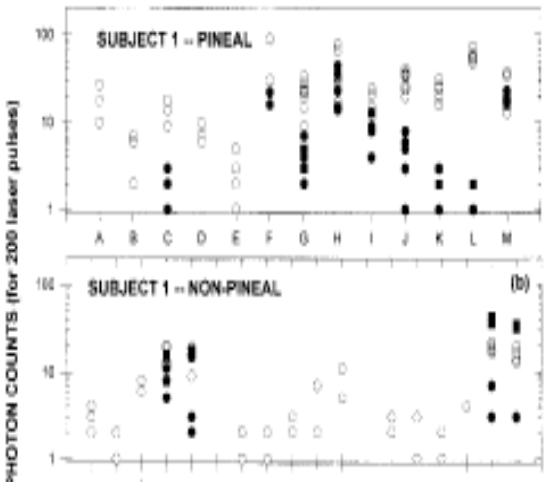


Fig. 1. SHG measurements on tissues from Subject I. Different measurement locations are designated by letters in the abscissa. The ordinates give the number of photon counts detected by the photomultiplier during the operating period of the laser.

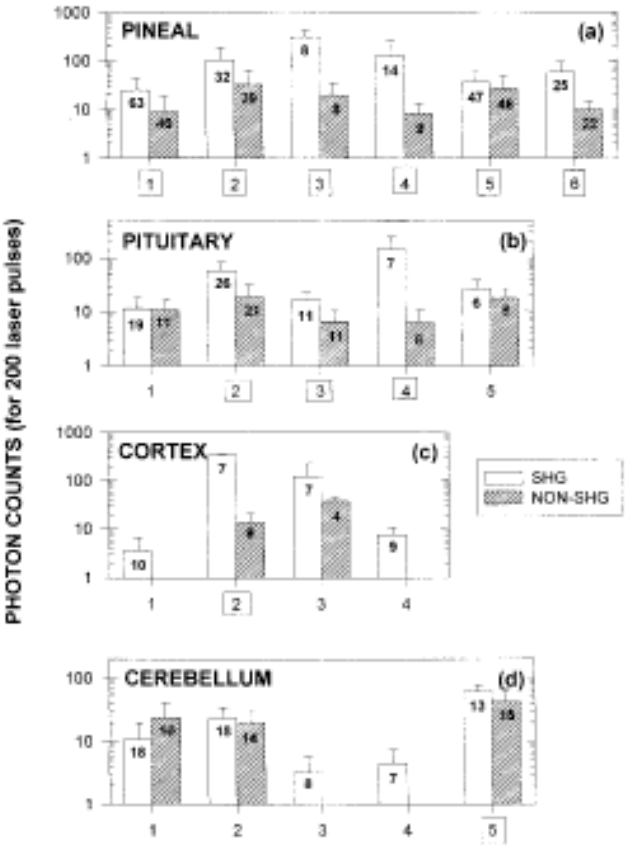


Fig. 2. SHG results on all of the available tissues from all of the subjects. The numbers in the abscissa identify the subjects. The ordinates give the number of SHG photon counts detected by the photomultiplier during the operating period of the laser. The numbers printed on the bars are the total number of measurements used in calculating the means. Other details of the figure are given in the text.

with the number of counts detected at other wavelengths (based on an unpaired Student's t-test at the $P < 0.05$ level).

Fig. 1 shows multiple measurements at different arbitrarily selected locations on tissues from Subject 1. The different measurement locations are designated by letters in the abscissa. The ordinate is a logarithmic scale which gives the number of SHG photon counts detected by the photomultiplier during 200 laser pulses. The number of photon counts at 532 nm are shown by open symbols and the numbers in the 515—520 and 540—550 nm windows by filled symbols. According to the criteria stated above, SHG was observed at a number of locations in the pineal tissue, but at no location in the other three types of tissues.

Fig. 2 illustrates SHG measurements on tissues from all six subjects. The numbers in the abscissa are the identification numbers of the subjects. Pineal tissues from all six subjects were examined, but some of the other tissues were not available for all of the subjects. The ordinates give the number of photon counts detected by the photomultiplier during the operating period of the laser. The bars show the mean ± standard deviation (SD) of the photon counts on all of the tissue samples from a given organ type for a specific subject. The total number of measurements used in calculating the mean is shown by the number printed on the bar. The unfilled bars indicate the pulses measured when the monochromator was set at 532 nm and the shaded bars show the pulses for settings 10—15 nm above or below 532 nm. An unpaired Student's *t*-test was used to determine if the means of the number of pulses detected at the 532-nm SHG wavelength differed from the number detected at other wavelengths. A box around the sample designator denotes a statistically significant difference *(P* <0.05, *t*-test). It should be noted that the ordinates are logarithmic scales. This is necessary to show the data clearly but it makes it difficult to judge statistical significance by a qualitative visual assessment.

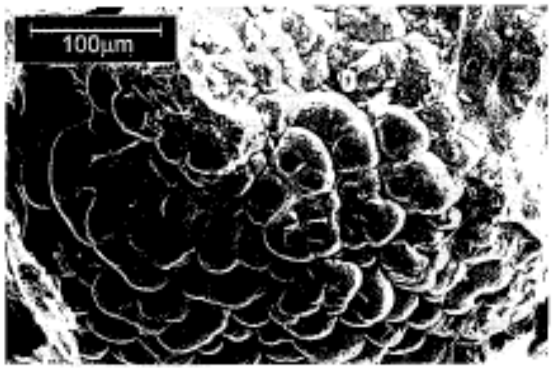


Fig. 3. SEM photograph of the mulberry-like structures in the pineal gland.

Statistically significant levels of SHO were found in all six pineal samples; three of the five pituitary, one of the four cortex and one of the five cerebellum also showed SHG at significant levels.

Four of the pineal samples were examined using SEM and three different types of crystalline structures were observed. Fig. 3 shows the mulberry-like structures observed by others [6,12—14]. EDS analysis of these structures gave Ca/P ratios of 1.8 to 1.9, somewhat higher than those observed by Bocchi and Valdre [5]. The second type

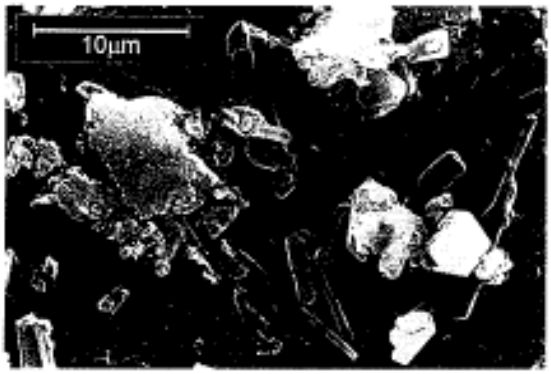


Fig. 4. SEM photograph of the faceted crystalline structures in pineal glands.


Fig. 5. SEM photograph of the faceted crystalline structures with unusual compositions in pineal glands.

resembled faceted single crystals (Fig. 4), a structure not reported previously. The large dimensions of these crystals were in the range 3—15 μm. Ca/P ratios were about 1.7, similar to those observed in bone hydroxyapatite [15].

Crystals with unusual compositions were observed in some of the pineal gland tissues. An SEM photograph of one is shown in Fig. 5. The composition of the long crystal in the center of the photograph was: 3.4% Al, 32.9% Si, 1.3% Cl, 10.4% K, 2.5% Ti and 9.5% Zn (calculated on an atomic basis and only including elements with an atomic number greater than 11). In particular, the aluminum was unexpected.

Three pituitary tissues and one each of cortex and cerebellum were examined with SEM, but no crystalline materials were found with the exception of one crystal in a pituitary sample.

No SHG was observed in the ashed pineal material. X-ray diffraction analyses showed that the material was completely amorphous suggesting that the crystalline structures which produce SHG were destroyed by heating. EDS analyses yielded compositions of 5.3—8.0 at% Al, 29.8—31.4 at% P, 6.7—14.9 at% S and 48.4—55.5 at% Ca.

Because of the unusually high levels of aluminum in the crystals shown in Fig. 5 and in the ashed pineal material,

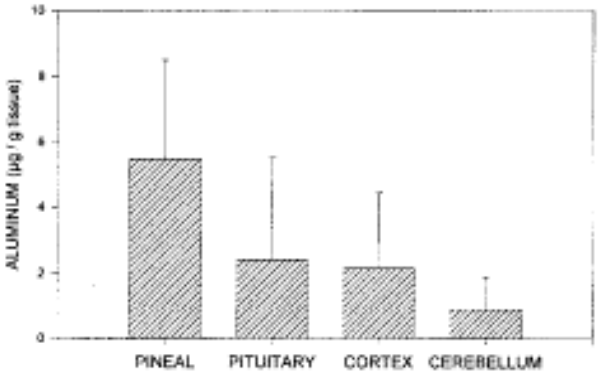


Fig. 6. Aluminum contents of brain tissues (mean ± SD). The results are expressed per unit weight of dried tissues. Four samples of each tissue were examined.

atomic absorption measurements were made on brain tissues from additional subjects to confirm the initial observations. The same four types of tissues from different sources were examined. The results are shown in Fig. 6. The concentrations of aluminum in the pineal gland are markedly higher than in the other tissues.

## 4. Discussion

The SHG results show that the pineal gland definitely contains noncentrosymmetric material which, according to crystallographic symmetry considerations [7], is piezoelectric. SHG detection does not permit determination of quantitative piezoelectric and other material constants. Piezoelectric crystals were detected throughout the human pineal gland (Fig. 1) in all subjects examined (Fig. 2).

The variation in the magnitude of the SHG responses (Fig. 1) is due to the nonuniform crystal distribution and variation in crystal size. A single crystal of a noncentrosymmetric material will show SHG, the magnitude of which depends on the crystal composition and size, and the alignment of the crystal axes to the laser beam [11,16]. If a pure noncentrosymmetric powder specimen is examined, the large number of grains and the randomness of their sizes and orientations with respect to the laser beam will ensure that an average SHG signal will be observed from different positions in the sample. In the biological tissues studied, the number of crystals in any area which is the size of the laser spot was low, their crystal sizes were small and relatively few crystals were properly aligned to give an SHG signal. These factors resulted in considerable variation in SHG magnitude from point to point. A large variation often occurred when the laser beam impingement point was moved only a few micrometers, a consequence of the small size of the crystals.

Significant SHG responses were observed in five of 14 non-pineal tissues examined (Fig. 2). The observed frequency appears to be too high to be accounted for on a statistical basis (failure to control for a familywise error), but the issue was not resolved in this study. We could not conclude, therefore, whether piezoelectricity also occurs in nonpineal tissue. We did find, however, that piezoelectricity was significantly more likely in the pineal gland, compared with nonpineal tissue (6/6 compared with 5/14, $P < 0.05$, Fisher's exact test).

There were at least three classes of crystals observed in the SEM that might have been the source of the SHG response: (a) mulberry-like calcifications (Fig. 3); (b) non-mulberry-like calcifications having a Ca/P ratio of 1.7—1.9 (Fig. 4); (c) noncalcium-containing crystals (Fig. 5). It is also possible that the SHG response was produced by small crystals located below the surface in the SEM views, but which were accessible to the laser beam during the SHG determinations. The first two kinds of crystals are chemically similar to bone mineral, which is not piezoelec

tic [17], perhaps suggesting that they were not the source of the SHG response. On the other hand, the crystal structure of bone mineral and pineal calcifications is not well known, and it is possible that small changes in crystal structure could result in the appearance of noncentrosymmetry, hence piezoelectricity. With the exception of one location, the nonpineal tissues contained no crystals visible in the SEM. Thus, if the SHG determinations involving the nonpineal tissues (Fig. 2) are interpreted as negative, then it could be concluded that there is a strong correlation between the presence of crystals and piezoelectricity.

Aluminum was consistently observed in the pineal glands using independent methods of measurement (EDS on powder, EDS on single crystals, atomic absorption), indicating that the element is consistently present in the human pineal gland. Its relationship to the piezoelectric property of pineal deposits, however, is unknown.

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