

Quantification of high pressure nervous syndrome (HPNS) tremor in the guinea pig

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Gruenau, S. P., and M. J. Ackerman. 1978. Quantification of high pressure nervous syndrome (HPNS) tremor in the guinea pig. *Undersea Biomed Res.* 5(1): 95-104.—Previous studies have demonstrated two tremorgenic systems that involve separate brain mechanisms and exhibit different peak frequencies. One system (the thalamo-cortical) generates low frequency (4-8 Hz) tremor; the other (the olivo-cerebellar) produces high frequency (10-18 Hz) tremor. Based on this evidence, the present study focused on determining whether one or both of these tremor systems is involved in the high pressure nervous syndrome (HPNS). Specifically, the concern was to identify and to quantify amplitude and frequency characteristics of HPNS tremor in 8 guinea pigs breathing helium-oxygen during compression (40 ft/min) in a chamber dive to 61.6 ATA (2000 fsw) with a bottom time of 1 h. Rectal temperature was recorded and maintained at $39^{\circ}\text{C} \pm 1^{\circ}$. Leg tremor was recorded by magnetic inductance and stored on magnetic tape for power spectral analysis. Frequency histograms of the tremor data revealed development of a biphasic response. From surface to about 31.3 ATA (1000 fsw), a low-power, single, 4- to 6-Hz component was evident, which resembled fine or moderate tremor. Between 34.3 ATA (1100 fsw) and 61.6 ATA, a 12- to 18-Hz component emerged abruptly with a dramatic increase in power, which reflected coarse, uncontrollable tremors. In the first 5 to 10 min after the animals arrived at maximum pressure, relative power of the high frequency component dropped to and remained near base-line levels. These results support the hypothesis that HPNS tremor consists of two components and possibly two separate tremor systems.

power spectra analysis
hyperbaric pressure

Neuromuscular tremor is one of the first peripheral manifestations of the high pressure nervous syndrome (HPNS). It severely interferes with diver performance and safety (Bachrach and Bennett 1973). In simulated dives under slow, helium-oxygen compressions, human subjects have displayed normal tremor when maximum depth was held to 19.2 ATA (600 fsw). Under the same conditions, human subjects have shown a consistent increase in tremor magnitude and tremor frequency ranging from 3 to 13 Hz when the pressure level exceeded 31.3 ATA (1000 fsw) (Bachrach and Bennett 1973; Hunter and Bennett 1974; Bennett 1975; Berghage, Lash, Braithwaite, and Thalmann 1975; Rostain and Naquet 1975; Spencer, Find-

ling, Bachrach, and Karreman 1976). Whether this frequency shift in HPNS tremor indicates different neural mechanisms has not been determined.

That frequency is an important factor in determining the neural mechanisms of tremor has been demonstrated in a series of elaborate neurophysiological studies (Llinas and Volkind 1973; Lamarre 1975). These studies isolated two anatomically separate systems, each capable of evoking peripheral tremor. One system involves the thalamo-cortical loop responsible for generating low-frequency tremor of 4 to 6 Hz; a second system involves a rubro-olivo-cerebellar circuit responsible for producing a higher-frequency tremor of 7 to 12 Hz. Based on this evidence, the aim of the present study was to determine the frequency and amplitude characteristics related to HPNS tremor in the guinea pig.

METHOD

Eight male guinea pigs (*Cavia porcellus*—NMRI: [HA] CV) weighing between 300 and 400 g were housed singly under a rigid 12-h light, 12-h dark cycle. Food and water were allowed ad lib until the time of entry into the recording chamber. Compression was begun between 1300 and 1400 h.

Chamber and recording conditions

Guinea pigs were exposed, three at a time, in a Bethlehem Model 1836 10-HP chamber with a volume of approximately 170 liters. During the experiment each animal was fitted with a rigid neck restraint (which allowed for freedom of leg and body movement) and placed in a Plexiglas enclosure (23 cm × 23 cm × 18 cm). Leg tremor was recorded by a method described by Dill, Dorman, and Nickey (1968) in which magnetic inductance served as the signal generator. Briefly, a small, lightweight magnet was taped to one foreleg of the guinea pig. The animal was then positioned within the Plexiglas enclosure over a coil of wire placed below the floor. Signals generated in the coil by magnetic induction were amplified, filtered (0.3- to 30-Hz bandpass filter), and read directly into the A/D inputs of a computer. Rectal temperatures were measured by a Yellow Springs Instrument thermistor probe, Series 401, and recorded every 50 ft until a maximum pressure of 61.6 ATA (2000 fsw) was reached; they were then recorded every 15 min while the subjects were at maximum pressure. Using a procedure modified from Stetzner and De Boer (1972), we maintained rectal temperature at $39^{\circ}\text{C} \pm 1^{\circ}$ by gradually increasing chamber temperature from 27°C at surface to 36°C during compression to 61.6 ATA; it was kept at this level for a bottom time of 1 h.

Pressurization

Predive 20-min recordings of forelimb movement from each animal were made to establish a surface base line. Thereafter, 8 guinea pigs, in groups of 3, were compressed with oxygen to 1.3 ATA (10 fsw), then with helium to 2 ATA (33 fsw). After a 5-min stop, helium compression continued to 61.6 ATA at the rate of 1.21 atm/min (40 ft/min). Oxygen was adjusted to 0.83% (0.51 ATA) upon reaching 61.6 ATA, as measured with a Beckman Model F-3 paramagnetic oxygen analyzer. Time at 61.6 ATA was 60 min.

Data analysis

Tremor data, in 10-s segments, along with chamber depth and time-of-day information, were input on-line to a digital computer, which displayed the data on a cathode-ray oscilloscope. If

no data artifacts were noted, spectral analysis (Cooley and Tukey 1965) was performed and the results were stored on digital magnetic tape. This tape, indexed by pressure and time, could later be searched for specific spectra of interest. We accomplished statistical comparisons between spectra by using an analysis of variance with repeated measures (BMDP 2 V) program (Dixon 1975). These statistical methods were supplemented by continuous strip-chart recordings of the tremor data and visual inspection of guinea pig motor activity.

RESULTS

Frequency and amplitude characteristics of HPNS tremor in guinea pigs compressed to 61.6 ATA on helium revealed the development of a biphasic response. To illustrate this tremor developmental sequence, we show representative power spectral histograms derived from compression data, bottom-time data, and their corresponding analog tracings from four animals (Figs. 1 and 2). The following is a description of the tremor characteristics during compression and during 60 min at 61.6 ATA.

HPNS tremor development during compression

Between surface and 31.3 ATA, no visible sign of tremor was evident and power spectral analysis of guinea pig motor activity yielded a low-power, low-frequency (3–6 Hz) spectrum (Figs. 1A, 2A). Corresponding analog tracings showed a low-amplitude, low-frequency oscillation interspersed with movement artifact (Figs. 1B, 2B). From 31.3 ATA to 43.4 ATA (1400 fsw), a second frequency component (12–18 Hz) emerged, rapidly gained in power, and eventually dominated over the low-frequency signal. When high-frequency tremor first appeared, visual inspection verified that the subject was moderately tremulant, mainly in the fore and hind limbs. The 12- to 18-Hz tremor continued to dominate the remainder of compression to 61.6 ATA; however, in most subjects the maximum amplitude and relative power of this component diminished moderately before reaching final depth. The corresponding analog tracings supported this finding (Figs. 1B, 2B). In the final 19.2 to 25.2 atm (600–800 ft) of compression, tremor had developed into coarse, uncontrollable shaking of the entire body.

To determine whether differences in tremor intensity during compression were depth- and/or frequency-dependent, we subjected the data to a three-way (depth $\times$ frequency $\times$ subjects) factorial design. The frequency spectra derived from data obtained at surface, and at even 4.03 ATA (100-fsw) pressures from 8 animals, were divided into 3 bands (1–10, 11–20, and 21–30 Hz). The three-way analysis of variance with repeated measures indicated significant differences in pressure ($F = 7.04$, $df = 10/176$, $P < 0.001$) and subjects ($F = 3.47$, $df = 7/176$, $P < 0.002$) as a function of relative power of the tremor signal. Moreover, the effect of pressure level was found to be dependent upon individual subject variability ($F = 3.70$, $df = 70/176$, $P < 0.001$). As expected, the difference in intensity of the tremor among frequency bands was highly significant ($F = 162.99$, $df = 2/352$, $P < 0.001$), as was the interaction between frequency and pressure ($F = 10.17$, $df = 20/352$, $P < 0.001$). For this reason, Scheffé contrasts among the 3 frequency bands and 11 pressure levels were made. Unexpectedly, tremor intensity in the 1- to 10-Hz band remained unchanged for all pressure levels. Yet in the 11- to 20-Hz band, intensity comparisons made between the first 4 pressure levels (surface, 7.1 ATA [200 fsw]; 13.1 ATA [400 fsw]; 19.2 ATA [600 fsw]) and the last 2 pressure levels (55.5 ATA [1800 fsw] and 61.6 ATA [2000 fsw]) were significantly different (see Table 1). Other significant differences were noted in the 21- to 30-Hz band, i.e., tremor intensity at surface, 7.1 ATA, 13.1 ATA, 19.2 ATA, and 25.2 ATA were different from that at 61.6 ATA (see Table 1).

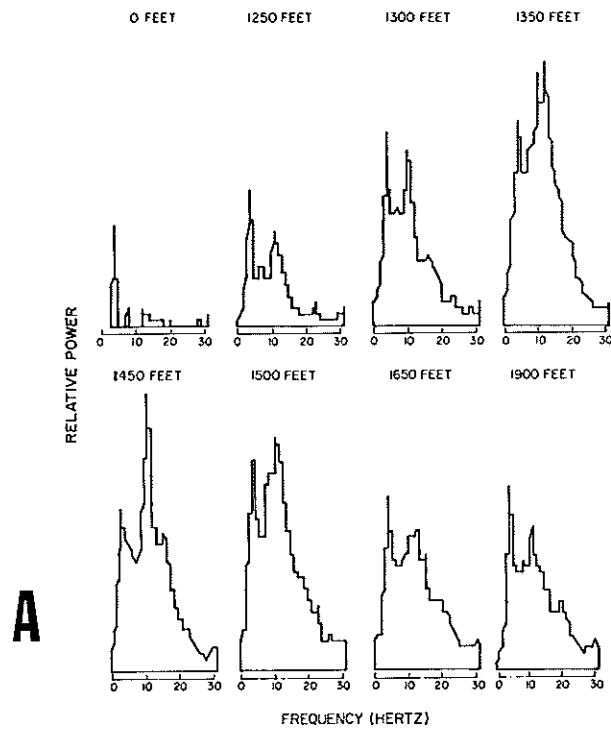


Fig. 1A. Power spectral histograms of tremor for 1 subject at exposure levels when major power and frequency changes occurred during compression. Note power shifts of frequency components in 3- to 6-Hz and 12- to 18-Hz bands.

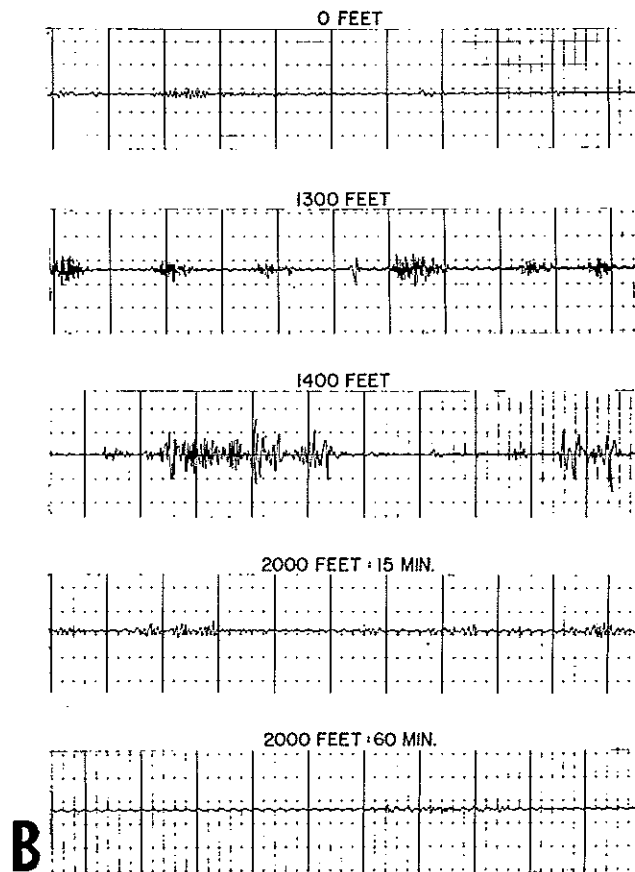


Fig. 1B. Representative analog tremor of forelimb for same subject sampled during compression and time spent at maximum depth (61.6 ATA). Amplitude scale is same for all tracings. Time between major divisions is 1 s.

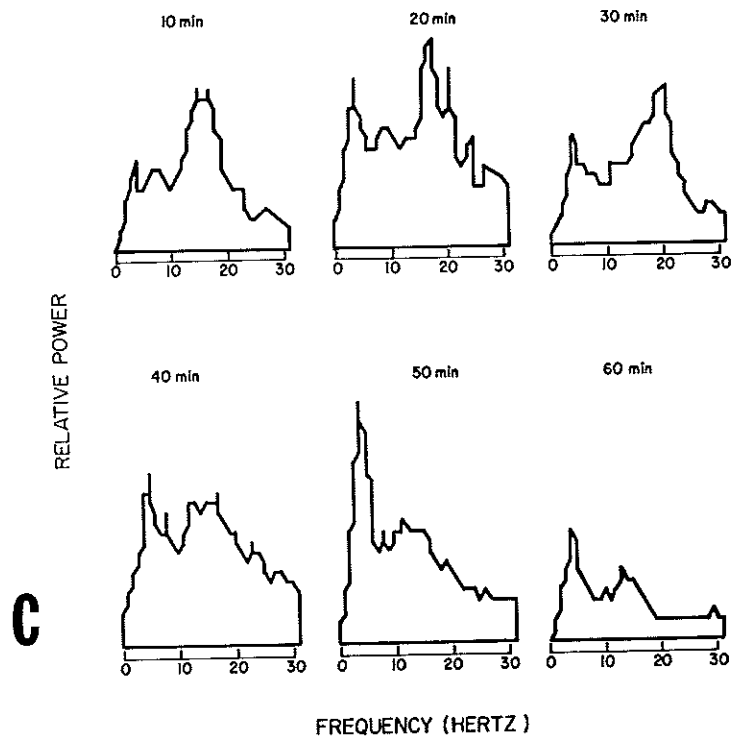


Fig. 1C. Power spectral histograms of tremor for same subject recorded every 10 min (from left to right) for 60 min at 61.6 ATA. In each subject, power shifted from high-frequency component to low-frequency component by end of bottom time.

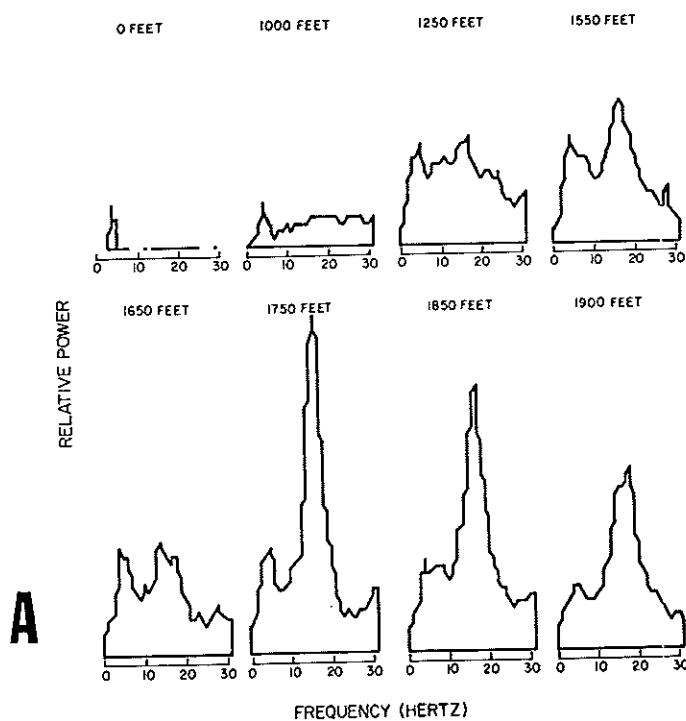


Fig. 2A. Power spectral histograms of tremor for 1 subject at exposure levels when major power and frequency changes occurred during compression. Note power shifts of frequency components in the 3- to 6-Hz and 12- to 18-Hz bands.

TABLE 1
SCHEFFÉ CONTRASTS OF HPNS TREMOR INTENSITY DIFFERENCES BETWEEN
PRESSURE LEVELS IN TWO FREQUENCY BANDS

Pressure	Surface	7.1 ATA	13.1 ATA	19.2 ATA	25.2 ATA
10–20 Hz					
55.5 ATA	<0.005	<0.005	<0.03	<0.07	NS
61.6 ATA	<0.004	<0.004	<0.03	<0.05	NS
21–30 Hz					
61.6 ATA	<0.007	<0.009	<0.06	<0.04	<0.09

Values are significant values; all *P* values are less than the values shown for each pressure.

HPNS tremor at 61.6 ATA

Tremor recording continued for 1 h after reaching 61.6 ATA, and analyses were made at 5-min intervals. Each animal demonstrated within the first 5 to 10 min of bottom time an immediate decrease in intensity or relative power of the 11- to 20-Hz component of tremor (Figs. 1C, 2C). After 60 min at bottom, tremor magnitude was at or near surface levels, with little or no evidence of tremor in the 11- to 20-Hz range. Visual inspection of the animals in the last 30 min of bottom time showed sporadic tremor confined to the limbs. Analysis of variance

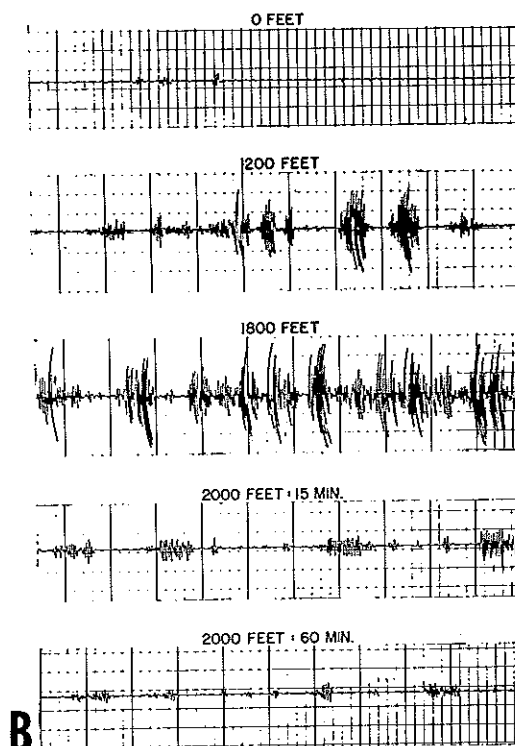


Fig. 2B. Representative analog tremor of forelimb for same subject sampled during compression and time spent at maximum depth (61.6 ATA). Amplitude scale is same for all tracings. Time between major divisions is 1 s.

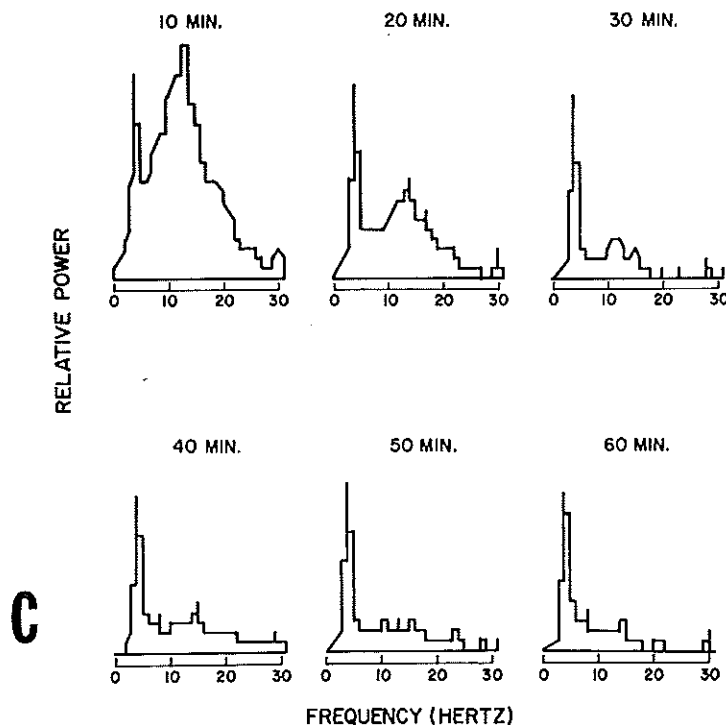


Fig. 2C. Power spectral histograms of tremor for same subject recorded every 10 min (from left to right) for 60 min at 61.6 ATA. In each subject, power shifted from high-frequency component to low-frequency component by end of bottom time.

indicated significant differences due to frequency as a function of time ($F = 8.86$, $df = 22/384$, $P < 0.001$). Based on this result, Scheffé contrasts among the 11 time intervals within each frequency band revealed no differences in the 1- to 10-Hz and 21- to 30-Hz bands; however, in the 11- to 20-Hz band, tremor intensity was significantly greater in the first 10 min than in the last 10 min of the bottom time ($P < 0.04$ and 0.03 , respectively).

DISCUSSION

This study demonstrated the feasibility and utility of applying quantitative techniques to the analysis of HPNS tremor in an animal model. Power spectral analysis, in particular, rendered new information regarding amplitude and frequency characteristics of HPNS tremorgenesis. It was shown, for example, that an association existed between two tremor frequencies and HPNS in the guinea pig. Frequency histograms revealed that a low-power, 3- to 6-Hz component of limb tremor predominated during compression from 0 to 31.3 ATA. Yet in compression from 31.3 ATA to 61.6 ATA, this 3- to 6-Hz component was overshadowed by the emergence of a 12- to 18-Hz spectral component, which peaked and remained high in relative power. It was during this period between 31.3 and 61.6 ATA that the guinea pigs displayed coarse, uncontrollable tremor of the entire body. Our results are in agreement with those of others in relation to the onset, intensity, and duration of HPNS tremor in rodents (Dossett and Hempleman 1972; Brauer 1975).

The double frequency response of HPNS tremor, however, has not been reported previously in animals. Rostain (1973) recorded electromyographic (EMG) activity from the forelimb

of the monkey under nitrogen-oxygen, helium-oxygen, and hydrogen-oxygen at various pressures and durations of exposure and found postural or intentional tremor in the 10–12 Hz range only. Direct recording from tremor generating sites are needed to resolve whether HPNS tremor involves more than one frequency component.

If a central mechanism can be assumed, then the biphasic frequency response to high pressures would suggest that two tremor systems may be involved in HPNS. Based primarily on the work of Lamarre (1975), one could conclude that the 12- to 18-Hz component may represent HPNS tremor activity generated from the rubro-olivo-cerebellar complex, whereas the lesser involved, low-frequency component (3–6 Hz) may represent activity of the thalamo-cortical loop of the CNS (Fig. 3). That the olivo-cerebellar system may be more responsible than the thalamo-cortical system for the generation of HPNS tremor has been partially supported by the work of Brauer (1975) and his colleagues, in which electrical activity of the caudate nucleus, a part of the thalamo-cortical system, failed to show significant changes prior to convulsive seizures. Brauer concluded that incomplete cerebellar lesions in rats did not alter HPNS tremor threshold. This conclusion may be explained by Lamarre and Dumont's (1972) finding that cerebellectomy in cats or monkeys not only prevents the occurrence of fast (7–12 Hz) tremor, but it also induces spontaneous (4–6 Hz) postural tremor.

It should be pointed out that other mechanisms besides direct CNS mediation may be responsible for generating HPNS tremor. One theory holds that normal rest tremor is ballistocardiographic (BCG) in origin (Brumlik 1962). But it would appear unlikely from our data that a BCG component could solely account for both spectral peaks of tremor occurring simultaneously, because the BCG frequency is based upon the cardiac systole. Another possible mechanism related to tremor production could be that the increase in chamber temperature

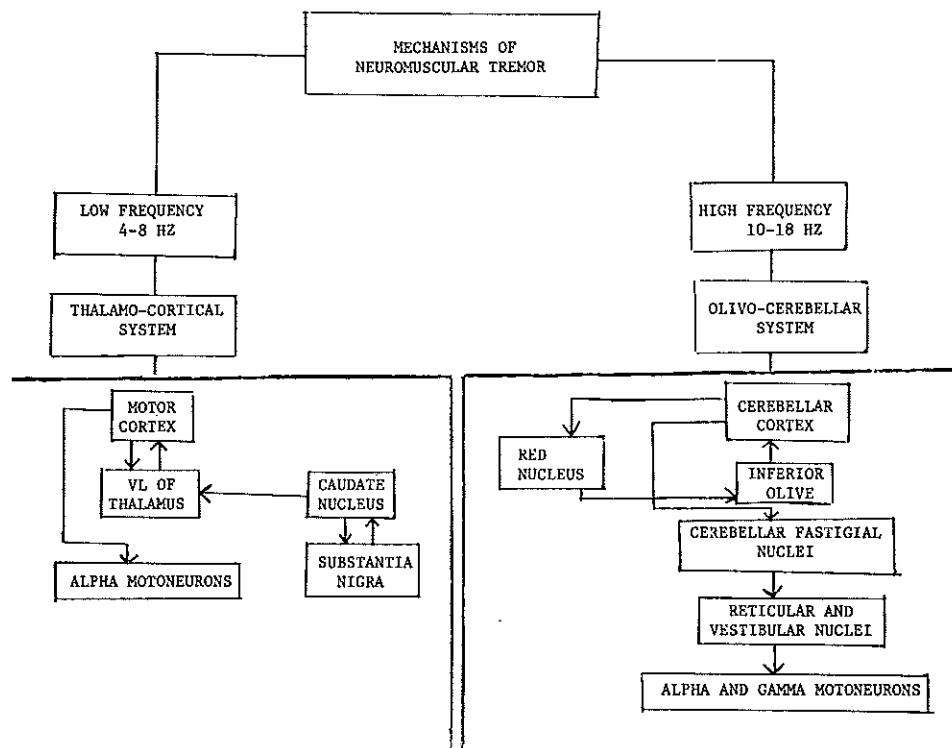


Fig. 3. Diagrammatic model of 2 tremor mechanisms. (Adapted from Lamarre (1975))

during compression, which was required to maintain core temperature, may have lowered the threshold for the onset of shivering, a condition observed in nonhyperbaric environments (Lomax 1970).

Naval Medical Research and Development Command, Research Task No. MR041.01.03-0148. The opinions and assertions contained herein are the private ones of the writers and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large. The experiments reported herein were conducted according to the principles set forth in the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council, DHEW, Pub. No. (NIH) 74-23. The support of the editorial staff of the Behavioral Sciences Department, Mary M. Matzen and Doris N. Auer, is gratefully acknowledged.—*Manuscript received August 1977; revision received December 1977.*

Gruenau, S. P., and M. J. Ackerman. 1978. La quantification du tremblement du syndrome nerveux des hautes pressions chez le cobaye. *Undersea Biomed. Res.* 5(1): 95-104.—Des études antérieures ont mis en évidence deux systèmes trémogènes qui correspondent à des mécanismes cérébraux distinctes et qui possèdent des fréquences maximales différentes. La première, thalamo-cortical, est à l'origine d'un tremblement à fréquence basse (4-8 Hz); l'autre, olivo-cérébelleux, détermine un tremblement à fréquence haute (10-18 Hz). À partir de ces données, nous avons essayé de spécifier la participation des deux systèmes dans le syndrome nerveux des hautes pressions (SNHP) en identifiant et en quantifiant l'ampleur et la fréquence du tremblement du SNHP chez 8 cobayes. Les animaux respiraient un mélange He-O₂ au cours de la mise en pression (40 pieds/minute) d'une plongée en caisson à 61, 6 ATA (2000 fsw); avec une heure de séjour au fond. La température rectale a été enregistrée et maintenue à 39°C ± 1°. Le tremblement a été enregistré au niveau de la jambe par inductance magnétique, sur bande magnétique pour permettre l'analyse électronique du spectre. Des histogrammes de la fréquence, basés sur ces résultats, ont mis en évidence une réponse biphasique. Du surface jusqu'à 31, 3 ATA (1100 fsw) environ, on a constaté un composant unique, à force basse (4-6 Hz) associé à un tremblement modeste ou moyen. Entre 34, 3 ATA (1100 fsw) et 61, 6 ATA, un composant est apparu tout d'un coup, avec une force beaucoup plus grande, associé à des tremblements importants, incontrôlables. Pendant les premières 5-10 min à fond, la force relative du composant à fréquence haute est redescendue à la valeur de base. Ces résultats renforcent l'hypothèse que le tremblement du SNHP est déterminé par deux éléments, et peut-être par deux systèmes différents.

analyse du spectre
pression hyperbare

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