normal-sized fibers scattered singly, or in groups, were found. These fibers retained a primitive appearance with a myotubal structure and centrally placed nuclei. Intramuscular nerve bundles were prominent (when stained with hematoxylin and eosin), but further examinations will be necessary to evaluate the structural integrity of the axones and nerve terminals.

These histological findings are consistent with denervation atrophy and degeneration (*14*), but occur on a greatly accelerated scale in the chick embryo, as compared with mature animals. Although skeletal muscles are already innervated in embryos of 7 days' incubation (*15*), such as were used in the present experiments, a similar picture has been described for chick embryo limb muscles which have never received primary innervation (*16*).

Theoretically, one cannot distinguish with absolute certainty between denervation atrophy and disuse atrophy of muscle by purely morphological criteria (*17*). However, the rapid appearance of atrophy, fat accumulation, and subsequent fiber degeneration favor denervation atrophy. In the present experiments, atrophy and fat accumulation were well advanced by 1 week after the injection of toxin, strongly suggesting the effects of denervation. The presence of even a few large but immature muscle fibers remains problematical.

These results are offered as preliminary evidence favoring a "trophic" influence of neural acetylcholine release on skeletal muscle. Further studies with the chick embryo confirming the specific physiological action of botulinum toxin, and outlining the pattern of disuse atrophy, are needed to support this hypothesis. Undoubtedly, botulinum toxin will prove to be a powerful tool for exploring the trophic role of acetylcholine release on other aspects of embryonic development.

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15 June 1964

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Electroencephalographic Correlogram Ratios and Their Stability

Abstract. *Autocorrelations of electroencephalograms can be reduced to ratios of estimated power among distinguishable parameters of typical tracings: the dominant rhythm, background activity, abundance, and total power. These data reduction methods permit statistical evaluation of differences among experimental conditions, thus extending the usefulness of graphic correlograms in research. Ratios discriminate between two experimental conditions and two subjects, while showing stability over days.*

The potential usefulness of correlation analysis to research in electroencephalography has been described in several reports (*1*). In the few studies where it has been used, experimenters have typically depended upon visual inspection of the correlogram, counted dominant wave frequency, or measured phase shifts. It is proposed that additional data can be extracted from correlograms, can be reduced to terms which are meaningful conceptually and which are quantified approximations of significant parameters, and can be subjected to statistical analysis for the testing of hypotheses.

Inspection of a typical cyclic correlogram (Fig. 1) shows most obviously the period of the dominant wave pattern. (However, its precise determination cannot be made from the first cycle because wave length may be distorted in this region.) In addition, the display contains displacement, which is a function mainly of low-frequency waves and non-rhythmic components, identifiable as a lack of balance around zero voltage at zero time delay and restoration of balance through time thereafter. A third feature is decay in amplitude of successive cycles which is attributable to the fact that the electroencephalogram (EEG) has a continuous (or non-line) power spectrum with characteristics related to amplitude and phase modulation.

According to current theory, the EEG rhythm "represents massed synaptic potentials of apical dendrites of mainly pyramidal cells becoming synchronous and oscillating as fields of maximal amplitudes," and infant patterns develop into "more rigidly defined synchronized alpha rhythms of adult life" (*2*). In line with this concept, the term "dominant synchronized rhythm" (DSR) shall be used to refer to the typical cyclic pattern reflected in the correlogram. Since this dominant rhythm resembles a modulated sine wave of random amplitude and phase, and desynchronization of the EEG leaves a pattern resembling limited band-width noise, then the mathematical demonstrations of correlogram interpretation (*3*) are appropriate for a first approximation analysis. (i) A modulated sine wave of random amplitude and phase yields a cosine correlogram with an envelope which decays exponentially. (ii) Restricted bandwidth noise (with no dominant frequency) gives an exponential correlogram. (iii) The correlogram of mixed wave forms is equivalent to the sum of correlograms of the separate wave forms. For the analysis in this report, the exponential functions are to be treated as linear.

Figure 1 is a diagram of a typical EEG autocorrelation function and of the basis for quantification. Vertical axes *AG*, *BH*, and *CI* identify the decaying correlogram cyclic amplitude at the zero-, half-, and full-period points respectively. Displacement is represented by the line connecting the midpoints of the axes (points *DEF*).

Values to be used for analysis may be taken directly from the graphic display. The ordinate value of point *A* is a good estimate of the mean power in the EEG epoch analyzed, or its mean square amplitude. Point *D* best represents mean power in the non-dominant frequencies, and the difference between *A* and *D* is an approximation of the mean power in the DSR. The value of the mean power in the DSR is representative of the common observational term "abundance" (persistence and amplitude) of the dominant wave, since both of these factors must contribute to the zero-delay power term by the very nature of correlation analysis. The ratio of DSR power to total power provides an estimate of abundance, thus:

$$\text{Abundance ratio } (AR) = (A - D)/A \quad (1)$$

Since the estimate of power in components other than the DSR is represented by the *D* value, and since such activity in the EEG forms a background for the dominant rhythm, this estimate also may be assessed against total power in a complementary ratio, thus

$$\text{Background ratio } (BR) = D/A \quad (2)$$

To directly reveal the degree to which the DSR stands out against secondary rhythms and noise, abundance and background estimates may be related to each other in the following manner:

$$\text{Dominance ratio } (DR) = (A - D)/D \quad (3)$$

By a similar procedure, power terms may be taken from other delay points on the correlogram, for example, at one-cycle delay (*CF*). More precisely, such an estimate is the integrated product of corresponding phase points on successive pairs of DSR waves, the mean wave being utilized as the standard. A pure sine wave yields a correlogram without decay, hence the degree to which the *CF* value falls short of the *AD* value suggests the lack of wave-to-wave coincidence. Theoretically, such lack reflects the presence of superimposed, nondominant activity, or of generator instability. The first full-cycle delay point was chosen for analysis because it represents the smallest range over which the concept of generator stability is reasonably applicable.

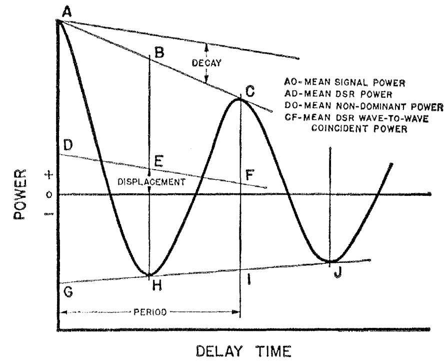

Fig. 1. Typical correlogram showing DSR cycles, displacement, and decay. Points *A* to *J* are used in the derivation of ratios. (DSR, dominant synchronized rhythm.)

Ratios are also appropriate for the assessment of coincident cycle-to-cycle power. When evaluated against mean power (*A*), the ratio reflects the degree to which the EEG epoch is synchronized wave-to-wave at the DSR, thus

$$\text{Synchronization ratio } (SR) = (C - F)/A \quad (4)$$

When evaluated against DSR power (*AD*), the cycle-to-cycle coincident power is interpreted by a ratio which reflects rhythm quality of those DSR waves which are present.

$$\text{Rhythm ratio } (RR) = (C - F)/(A - D) \quad (5)$$

These ratios express the relative power in various defined parameters of the EEG and permit the reduction of a graphic correlogram to statistics which may be further employed to assess subject differences or experimental condition effects. Data are presented in the remaining figures to demonstrate one such application of the ratios. Standard EEG recordings were made from the right occipital lobe of two subjects for five successive days for purposes of checking EEG stability. Appropriate controls for time of day and electrode placement were used. Recordings were made simultaneously in the graphic and magnetic tape media (*4*). For each subject, two representative 1-minute epochs were selected from the 2nd, 3rd, and 4th day for autocorrelation function analysis; one taken under eyes-closed and one under eyes-open conditions. Correlograms are shown in Fig. 2.

Figure 3 shows the calculated ratios for the two subjects, 3 days, and two conditions. It is evident that differences between conditions are striking in relation to the day-to-day consistency within a subject and condition. Since the subjects (males, age 24) were not selected for EEG pattern or stability, the data are suggestive of individuality in these ratios. In spite of daily variability in absolute correlogram amplitudes (Fig. 2) the obtained abundance and background ratios (*AR* and *BR*) indicate that the estimates of DSR power and non-DSR power show a remarkably constant daily ratio to total power within the epoch.

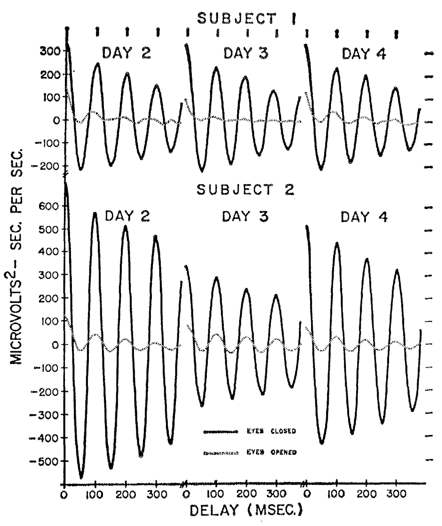

Fig. 2. Autocorrelograms of 1-minute epochs for two subjects, two conditions, and three consecutive days.

The remaining ratios for the eyes-closed condition [with the exception of the dominance ratio (*DR*) for the second subject] are almost as stable. More variability is shown under eyes-opened conditions, which may be related to the fact that no control was provided over the visual field or fixation. In no case does one subject's ratio overlap the range of ratios of the other subject under identical conditions, nor is there overlap between conditions for a subject. Experimental data of this kind, properly treated, could be subjected to analysis of variance or another appropriate statistical test. Furthermore, it is a simple matter to derive formulas for the determination of terms used in the ratios, when the numerical values of *A*, *C*, *H*, and *J* can be taken directly from the correlation analysis. With this procedure, computer analysis of correl-

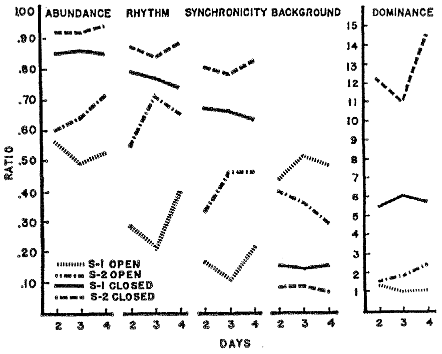

Fig. 3. Correlogram ratios for two subjects, two conditions, and three consecutive days. (S-1, first subject; S-2, second subject.)

ograms is possible in large-scale studies.

It must be emphasized that these ratios depend upon approximations of complex functions in the EEG. Theoretically at least, some of the estimates could be obtained as well from power spectrum analysis, and in many cases the power spectrum may be a useful adjunct in determining applicability or interpretation. There are probably some circumstances where the ratios can be applied only with considerable caution; for example when dominance is not clearly monorhythmic. For these reasons, further work is needed to provide information on the limits of their applicability, provide stronger validation, and perhaps to obtain the terms with greater mathematical elegance. Meanwhile, I have shown (*5*) that these ratios, unlike single absolute parameters such as frequency or amplitude, do distinguish degrees of arousal in human subjects.

In consideration of the importance placed upon desynchronization in activation theory (*6*), it is apparent that the method proposed in this paper should be of use. In discussing frequency analysis, Knott (*7*) said, "an instrument designed to complement *visual* analysis should not lead just to further visual analysis." Since the remark also applies to correlation analysis, the ratios are offered for their possible use as data reduction techniques.

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Reversible Cold Block of the Specialized Cardiac Tissues of the Unanesthetized Dog

Abstract. *The sinoatrial node and the bundle of His in intact, unanesthetized dogs were subjected to local cooling by means of surgically implanted devices. Impulse formation in the sinoatrial node could thus be suppressed at will and the heart rate regulated within a physiological range by an electrode driving the atrium. Reversible blockage of atrioventricular conduction was easily induced on cooling the region of the bundle of His.*

Local cooling of the specialized conduction system of the heart results in reversible suppression of impulse formation and impulse transmission (*1*). Hypothermia decreases the spontaneous activity of pacemakers (*2*), decreases conduction velocity in the atria and ventricles of mammalian hearts, and slows impulse propagation at the junction of the atrium with the atrioventricular node (*3*). A similar effect of local cooling has been noted with reference to impulse transmission in the central nervous system (*4*). The purpose of the study reported herein was to control heart rate and atrioventricular conduction in an intact unanesthetized animal. Local cooling of the sinoatrial node and of the bundle of His in the intact dog resulted in inhibition of impulse formation at the sinoatrial node and in varying degrees of blockage of atrioventricular conduction.

Two adult mongrel dogs were anesthetized with 30 mg of pentobarbital per kilogram of body weight, were intubated and kept under conditions of controlled ventilation. The chest was entered through the right fourth intercostal space and the heart exposed. In one animal a 30-cm length of fine silver tubing (inner and outer diameters, 0.165 cm and 0.178 cm, respectively) was wound into a tight flat coil about 2 cm in diameter (Fig. 1) and sutured to the right atrium over the region of the sinoatrial node. Polyethylene tubing (No. 200) was tightly fitted to each of the free ends of the coil. A pacemaker electrode was sutured to the right atrial appendage. The pericardium was closed loosely. The two lengths of polyethylene tubing, together with the electrode cable, were brought out of the chest at a point between the scapulae.

In a second dog, right atriotomy was performed under inflow occlusion. During this procedure, a 4-cm length of the silver tubing, bent into a "U," was sutured to the interatrial septum in the region of the bundle of His. One leg of the "U" was placed parallel to, and about 0.6 cm above, the septal leaflet of the tricuspid valve; the open end of the "U" was directed toward the coronary sinus (Fig. 1). Polyethylene tubing (No. 200), tightly fitted to each leg of the "U," extended outside the chest which was closed as before.

The dogs were permitted to recover from surgery for 5 days before records were taken. During each experiment the animal was sedated with morphine sulfate, 1 to 2 mg/kg. Electrocardiograms were recorded on a Sanborn polyviso. The sinoatrial node and the bundle of His were cooled by passing a mixture of 95 percent alcohol and carbon dioxide ice through the silver tubing. One end of the polyethylene tubing was attached to a suction pump while the other end was immersed in a reservoir of the cooling mixture. Passage of the cooling mixture (initially at −10° to −20°C) through the coil inhibited impulse formation at the sinoatrial node. After suppression of the sinus pacemaker, the heart was paced at various rates by means of the electrode attached to the right atrial appendage. The stimulus intensity was between 5 and 15 v and the stimulus duration 5 msec. To suppress atrioventricular conduction, the alcohol-carbon dioxide mixture, cooled to temperatures as low as −45°C, was drawn through the silver tubing. The temperature in the coil and "U" tube was varied by controlling the proportion of alcohol and carbon dioxide ice in the cooling mixture reser-

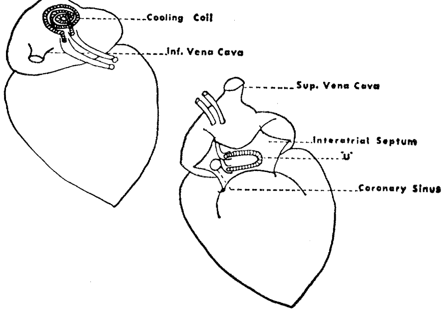

Fig. 1. Right aspect of heart showing cooling coil, sutured over the sinoatrial region (left) and bent into a "U" on the interatrial septum (right).