til well over 500 msec after the shock (at arrow). Since higher stimulus intensities (as well as multiple stimuli at low intensity) decreased the response latency to less than 50 msec, most of the delay is probably due to slow spread of the activity within the cord explant.

These experiments demonstrate the remarkable degree to which the bioelectric activities of cultured human, rat, and chick spinal cord tissues resemble complex CNS patterns *in situ*, even after months of isolation in vitro. A powerful new experimental approach is therefore available to supplement *in situ* studies of CNS function, especially those aspects concerned with deafferented or other types of neuronally isolated CNS regions (*9–11*). The complexity of the long-lasting excitatory phenomena evoked in cultured cord tissue suggests sequential activation through multiple chains of synaptically linked neurons (*12*). Other factors which may be responsible for repetitive neural activity *in situ* must also be considered here, such as sustained oscillation of local membrane potential, differential repolarization, and persistence of humoral transmitters (*9*). Further analysis of the bioelectric activities of cultured CNS tissue will be of great interest since a relatively small number of neural elements can be studied as an isolated, model nervous system, in a controlled chemical environment, during direct observation of cytologic details of the constituent neurons and glia. Furthermore, since the tissues can be explanted at early embryonic stages, correlative studies of neural structure and function can be made during critical stages of differentiation and maturation in vitro. This approach is being extended in current electrophysiologic experiments with long-term cultures of neonatal mouse cerebral neocortex where a high degree of structural and functional organization develops after explantation in vitro (*13*). It should be emphasized that the techniques used in the preparation and long-term maintenance of healthy CNS cultures require meticulous control over many laboratory procedures, but we believe that the research potentialities of this method warrant the required effort and expense (*14*).

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References and Notes

1. S. M. Crain, *J. Comp. Neurol.* **104**, 285 (1956).
2. W. Hild and I. Tasaki, *J. Neurophysiol.* **25**, 277 (1962).
3. A preliminary report of this work was presented at the 2nd *Ann. Meeting Am. Soc. for Cell Biol.*, San Francisco, 1962, Abstr., p. 36.
4. A. W. B. Cunningham, *Nature* **190**, 918 (1961); *J. Gen. Physiol.* **45**, 1065 (1962).
5. M. B. Bornstein, *Lab. Invest.* **7**, 134 (1958).
6. E. R. Peterson and M. R. Murray, *Am. J. Anat.* **96**, 319 (1955); M. B. Bornstein and M. R. Murray, *J. Biophys. Biochem. Cytol.* **4**, 499 (1958).
7. E. R. Peterson, M. B. Bornstein, S. M. Crain, *Excerpta Med.* **16** (Sect. 1), 813 (1962).
8. R. P. Bunge and M. B. Bunge, in preparation.
9. B. D. Burns, *Can. J. Biochem. Physiol.* **34**, 457 (1956); *The Mammalian Cerebral Cortex* (Arnold, London, 1958).
10. G. W. Stavraky, *Supersensitivity following Lesions of the Nervous System* (Univ. of Toronto Press, Toronto, Canada, 1961).
11. D. P. Purpura and E. M. Housepian, *Exptl. Neurol.* **4**, 377 (1961).
12. R. Lorente de Nó, *J. Neurophysiol.* **1**, 207 (1938).
13. S. M. Crain, *Trans. Am. Neurol. Assoc.*, in press (1963); ——— and M. B. Bornstein, *ibid.*
14. Aided by grants NB-03814 and NB-00858 and research career program award (NB-K3-2904) from the National Institute of Neurological Diseases and Blindness.

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Signal Analysis of Evoked Potentials Recorded from Cats during Conditioning

Abstract. *Correlation coefficients were computed between average response waveforms recorded from different brain regions of trained cats, before and after a specific stimulus acquired cue value. Application of signal-analysis techniques to the correlation matrix shows marked increase in the similarity between waveshapes evoked by that stimulus in sensory-specific and nonsensory-specific regions.*

Numerous reports (*1*) have described extensive changes in electrophysiological responses to an intermittent stimulus as it acquires informational significance by being used as the conditioned stimulus during behavioral training. Electrical activity in sensory-specific structures appears to reflect closely certain attributes of the actual physical stimulus, such as the frequency of a flickering light. During conditioning, the electrical activity in nonsensory-specific structures increases markedly in response to the conditioned stimulus. In cats trained to differentiate between two frequencies, the response of nonsensory-specific regions may or may not correspond to the actual stimulus. When the frequency correspondence is good, behavioral response tends to be appropriate to the intermittent conditioned stimulus actually present. When the correspondence is poor, behavioral response tends to be inappropriate. On such occasions, activity in nonspecific regions sometimes displays the frequency of the stimulus appropriate to the behavior displayed by the cat. On the basis of evidence from such studies, John and Killam (*2*) proposed that such discrimination behavior involved a comparator system, possibly localized in the cortex, which estimated the degree of congruence between the temporal patterns of electrical activity in sensory-specific and in nonsensory-specific structures of the brain.

During further work, the impression was gained that a number of diverse anatomical regions, which initially displayed appreciable differences in evoked responses to a particular stimulus, came to display markedly similar electrical responses as meaning was attached to that stimulus during conditioning. Similar observations have been described recently by Galambos and Sheatz (see *3*).

We devised a technique to obtain a quantitative estimate of similarity between electrical waveforms recorded from different brain areas and to describe the set of organized relationships inherent in the configuration of similarities.

A Mnemotron four-channel average response computer was used to obtain average response waveforms from a large number of electrodes ($N = 14$ to 34) chronically implanted into cats. Each average response computation was based on 200 iterations of the stimulus. In four cats, averages were obtained at various stages during the elaboration of differential avoidance response. At each behaviorial stage, N average response waveforms were obtained from each animal, one average from each electrode derivation. The waveforms were then read out, in digital form, from the average response computer.

The data processing was carried out on the I.B.M. 7070 digital computer at the University of Rochester Computing Center. The waveforms were normalized to equate the differences in signal strength. Cross-correlation coefficients (the Pearson product-moment correlation coefficient) were computed for all pairs of waveforms within a given stage of behavioral response. Correction for differences in latency of the evoked potentials had a negligible effect, because of the relatively low frequency of the waveforms. Distributions of these correlation coefficients, before

Table 1. Changes in principal factor regression equations from stage III to stage VI, in cat No. FC-9.

Site	Stage III				Stage VI			
	1st	2nd	3rd	4th	1st	2nd	3rd	4th
L.VIS*	0.877	−0.349	0.051	0.248	0.818	−0.143	−0.502	−0.106
R.AUD	0.782	0.493	−0.281	−0.050	0.897	0.221	0.210	−0.236
MOT	0.870	0.426	−0.075	−0.031	0.715	0.453	−0.048	0.191
CAUD	0.952	0.039	−0.238	0.066	0.973	0.184	0.010	−0.026
VA	0.683	−0.413	0.112	−0.397	0.964	−0.076	0.146	−0.060
CM	0.721	−0.435	0.219	−0.378	0.987	0.081	0.050	0.055
OT	0.745	−0.401	0.201	−0.404	0.856	0.309	0.291	0.111
RF	0.690	0.446	0.234	0.248	0.921	0.286	0.148	0.004
LG	0.717	−0.448	0.205	−0.387	0.046	0.879	0.043	−0.010
LG_B	0.690	0.164	0.216	−0.188	−0.674	0.274	−0.075	0.515
SC_B	−0.011	−0.750	−0.375	0.252	0.364	−0.812	−0.234	−0.084
RH	0.972	0.063	0.064	0.175	0.975	0.010	0.150	0.058
NR	0.967	0.115	−0.124	0.079	0.905	0.200	−0.327	0.038

* Abbreviations: *L*, left; *R*, right; Subscript B, bipolar recording (all others are monopolar); VIS, visual, AUD, auditory, and MOT, motor cortexes; CAUD, caudate nucleus; VA, ventralis anterior; CM, centre median; OT, optic tract; RF, mesencephalic reticular formation; LG, lateral geniculate; SC, superior colliculus; RH, dorsal hippocampus; NR, nucleus ruber.

and after flicker training, did not show any overall shift, although marked changes were observed in the correlation coefficients between particular structures. More marked shifts in the distribution of correlation coefficients have been reported by Livanov (*4*) and by Glivenko *et al.* (*5*). These workers used somewhat different computational procedures, but the fact that they observed waveshapes only at cortical loci may be significant.

The N waveforms may be thought of as a set of N signal vectors in a signal space. The correlation coefficient between two waveforms represents the cosine of the angle between the two corresponding signal vectors. By the method of principal factors, the dimensionality, K, of the signal space spanned by the set of N signal vectors was determined. An orthonormal set of basis vectors (factors) was derived which spanned that space. The dimensionality of the hyperspace was arbitrarily defined by the number of basis vectors which accounted for 97 percent of the communality, or variance, of the space.

A measure of the degree of coupling between waveforms in the signal set can be obtained by plotting the amount of communality, or variance (V_m), in the signal space, which can be accounted for as the number of dimensions (factors) increases. A highly organized set of waveforms with a marked degree of similarity, or nonindependence, will be characterized by a sharply rising curve, while a loosely coupled, relatively dissimilar set of waveforms will yield a more slowly rising curve. Figure 1 shows the function describing the response to a 4/S flickering light for a typical animal at two different stages of a complex training sequence: first, after avoidance conditioning to a *steady* stimulus, while the flicker is still neutral (stage III); and second, after the animal has acquired a differential avoidance response to two flicker frequencies (stage VI). This curve shows a somewhat greater steepness after flicker acquired cue value, and some decrease in the number of factors necessary to account for 97 percent of the variance in the waveforms. This tendency was slight and suggests that the average response waveforms evoked by flicker from some anatomical regions become more similar as flicker acquires a particular meaning (*6*).

The distribution of correlation coefficients and the dimensionality of the signal space are measures that reflect the *average* degree of relatedness among a large number of anatomical sites. Such analyses do not describe the details of the relationships between specific areas of the brain. However, further analyses can be implemented which will permit examination of such details.

It is possible to reconstitute each average response waveform as a linear combination of these factors, with appropriate weighting coefficients (factor loadings) for each component. Similarity between the loading coefficients for a given factor in the regression equations which reconstruct two waveforms indicates a common component in the two waveshapes.

Examination of the configuration of principal factor loadings presented in Table 1 shows that as flicker acquires cue value for a *previously trained* cat, a high degree of similarity in the waveshape of the evoked response to flicker appears between regions of the brain which are sensory-specific and nonsensory-specific: particularly the visual cortex, ventralis anterior, centre me-

Table 2. Changes in physiological factor regression equations from stage III to stage VI in cat No. FC-9. This table presents the factor loadings necessary to reconstruct the average response waveshapes in the various recording sites in terms of the physiological factors represented by the average response waveshapes in CM, LG, RH_B, $CAUD_B$, and NR_B. The accuracy of the reconstruction is indicated by the rms error. Note the change in these equations as the stimulus acquires cue value.

Site	Stage III						Stage VI					
	CM	LG	RH_B	$CAUD_B$	NR_B	rms reconstruction error	CM	LG	RH_B	$CAUD_B$	NR_B	rms reconstruction error
L.VIS*	0.310	−0.134	−0.129	−0.257	−0.664	0.388	0.748	−0.034	−0.164	0.019	−0.492	0.285
R.AUD	−0.049	0.014	0.440	0.803	−0.392	0.621	−0.982	0.044	0.269	0.049	−0.430	0.304
MOT	0.301	−0.090	−0.268	−0.647	0.100	0.690	0.625	0.192	−0.153	0.224	0.254	0.583
LG_B	0.606	−0.154	−0.317	−0.272	0.132	0.777	−1.016	0.837	0.465	0.268	−0.336	0.384
VA	1.397	−0.453	0.073	0.076	−0.034	0.306	1.081	−0.130	0.073	−0.177	−0.046	0.229
CAUD	0.609	−0.369	−0.022	−0.467	−0.298	0.641	0.969	0.084	−0.145	0.042	0.020	0.134
NR	0.526	−0.288	−0.154	−0.515	−0.220	0.633	0.729	0.155	−0.334	0.294	−0.107	0.340
OT	0.513	0.483	0.016	−0.024	0.020	0.199	0.725	0.392	0.036	0.161	0.052	0.312
SC_B	0.358	−0.509	0.500	0.444	−0.851	0.679	0.297	−0.799	−0.200	0.121	−0.191	0.347
RF	−0.184	0.105	−0.532	−0.670	0.023	0.669	0.867	0.094	−0.235	0.192	0.244	0.208
RH	0.370	−0.170	−0.342	−0.509	−0.282	0.521	0.996	−0.044	0.050	−0.042	0.003	0.230

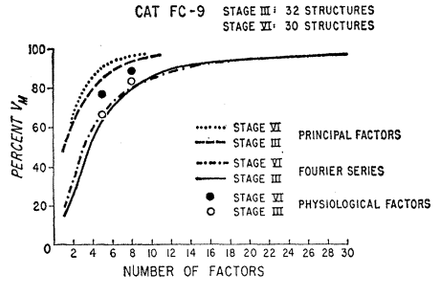

Fig. 1. Variance (V_m), plotted against the number of factors (abscissa) obtained from analyses by principal factors, physiological factors, and Fourier series. At stage III a conditioned avoidance response to the onset of steady light has been established, and the flicker is a neutral stimulus; at stage VI, a differentiated avoidance response has been established between two frequencies of flickering light.

dian, mesencephalic reticular formation, and the dorsal hippocampus. In three additional cats, some indication has been obtained that these relationships change during spontaneous errors or during blockade of performance after the administration of reserpine (*7*).

The reconstruction factors provided by the method of principal factors have no physiological significance but simply permit a concise and convenient representation of the waveforms. In further work, a reconstruction scheme based on factors chosen by physiological criteria was utilized. Selection of waveforms showing the highest loading on the first few principal factors allows the construction of a set of oblique physiological factors. Regression equations can now be calculated which describe the waveshape in many regions of the brain as linear combinations of the waveshapes in a small number of anatomical loci. Examination of such physiological regression equations suggests that as a stimulus acquires a particular meaning, the functional influence of the thalamic reticular formation is markedly enhanced. Table 2 presents regression equations which reconstruct, in terms of physiological factors, the same set of waveshapes for which a principal factor description is provided in Table 1. Details of the reconstitution of waveforms with these two techniques have been discussed (*7, 8*).

It is of interest to compare the degree of data reduction obtained from analyses by principal factors and physiological factors to that obtained from an analysis by Fourier series. A comparison can be made from the data in Fig. 1 where the variance (V_m) of the signal space accounted for by m factors is plotted against m for each of the three methods. By this measure, the analyses by both principal factors and physiological factors are more efficient than by Fourier series. Some degree of similarity of waveshape is to be expected due to constraints on the frequency response of the potentials. However, the greater efficiency of the analyses by principal factors and physiological factors suggests that some of the similarity is due to covariation of certain clusters of terms in the set of waveforms as described by Fourier series. This covariation may be due to functional relationships that exist between the anatomical sites from which the potentials were recorded.

As informational significance is attached to a stimulus by conditioning, the average potentials evoked by that stimulus in diverse brain regions acquire a marked similarity in waveshape. The observed correspondence between the time course of electrical activity in diverse brain regions, and the diminution in this correspondence with inappropriate performance, suggests that this reflects the organization of a functional system processing information about the stimulus. The data reported here are in support of the comparator hypothesis stated earlier. The observed waveshape correspondence between neural regions is interpreted to mean that the pattern of axonal discharges impinging as afferent input upon a region produces in that region a reflection of the macropotential waveshape in the region whence the axonal discharges originate. Changes in waveshape correspondence between regions are interpreted as changes in the effectiveness of the influence of certain regions upon others. The data analysis and reduction techniques outlined herein are applicable to a wide variety of other kinds of electrophysiological and neuropharmacological problems. A fuller account of these methods and of the result obtained has been presented recently and will shortly be published (*7, 8; 9*).

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References and Notes

1. F. Morrell, *Physiol. Rev.* **41**, 443 (1961); E. R. John, *Ann. Rev. Physiol.* **23**, 451 |1961).
2. E. R. John and K. F. Killam, *J. Nervous Mental Disease* **131**, 183 (1960).
3. R. Galambos and G. C. Sheatz, *Am. J. Physiol.* **203**, 173 (1962).
4. M. N. Livanov, *Intern. Congr. Physiol. Sci. 22nd, Leiden, 1962. Proc.* vol. I(II), p. 899.
5. E. V. Glivenko, T. A. Korol'kova, G. D. Kuznetsova, *Sechenov Physiol. J. U.S.S.R.* (English transl.) **48**(9), 1026 (1962).
6. Some of the average response waveforms which comprised the raw data for these analyses were provided by K. C. Corley, who obtained them in the course of his Ph.D. dissertation.
7. E. R. John, D. S. Ruchkin, J. Villegas, in *Sensory Evoked Response in Man* (New York Acad. Sci., in press).
8. D. S. Ruchkin, J. Villegas, E. R. John, in *Computers in Medicine and Biology* (New York Acad. Sci., in press).
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Ginkgophyton (Psygmophyllum) with a Stem of Gymnospermic Structure

Abstract. *A specimen identified as* Ginkgophyton (Psygmophyllum) *with a structurally preserved stem of gymnospermic organization has been collected from a late Devonian stratum in New York.*

The taxonomic position of a complex of Paleozoic genera consisting of flabelliform or cuneiform leaves, occasionally attached to branching axes, has been one of the most perplexing of paleobotanical problems for nearly a century. Included in this group, which ranges stratigraphically from Devonian to Permian, are *Platyphyllum, Germanophyton, Ginkgophyllum, Ginkgophyton, Psygmophyllum,* and, indeed, *Enigmophyton.*

In the most recent discussion of these genera, Høeg (*1*) provides an excellent summary of their morphology and an analysis of nomenclatural problems. As he emphasizes, *Ginkgophyllum* and *Psygmophyllum* were established for Upper Carboniferous and Permian species of distinctive morphology. Both, but especially *Psygmophyllum*, have also been used for the inclusion of Devonian species. Of the other genera, all of which are characteristically Devonian, *Enigmophyton* Høeg is the most completely known. *Enigmophyton superbum,* from the Upper Middle or Lower Upper Devonian of Spitsbergen, is reported to have a dichotomizing branch system bearing a secondary system of smaller lateral branches and sessile, flabelliform, dichotomously veined leaves up to 16 cm long and 12 cm wide (at the broadest point). These