17. V. R. Lingappa, J. R. Lingappa, R. Prasad, K. E. Ebner, G. Blobel, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2338 (1978).
18. W. M. Bonner and R. A. Laskey, *Eur. J. Biochem.* **46**, 83 (1974); R. A. Laskey and A. D. Mills, *ibid.* **56**, 335 (1975).
19. We thank Dr. W. Brill, University of Wisconsin, for providing *Rhizobium* strains SM3 to SM5, and Dr. J. Burton, Nitragin Co., Milwaukee, for *Rhizobium* strains 61A76 and 61A24. Supported by grants from the Rockefeller Foundation, National Research Council of Canada, Quebec Ministry of Education, and the Canada Department of Agriculture.

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Automatic Classification of Electroencephalograms: Kullback-Leibler Nearest Neighbor Rules

Abstract. *A prototypic problem in screening of electroencephalograms in the automatic classification of stationary electroencephalogram time series is treated here by the Kullback-Leibler nearest neighbor rule approach. In that problem, the category or state of an individual is classified by comparison of his or her electroencephalogram with those taken from other individuals in the alternative categories. The Kullback-Leibler nearest neighbor classification rules yield a statistically reliable estimate of the smallest possible probability of electroencephalogram misclassification with a relatively small number of labeled sample electroencephalograms. The automatic classification of anesthesia levels L1 and L3, respectively the anesthesia levels insufficient and sufficient for deep surgery, is treated by machine computation on the electroencephalogram alone.*

We have applied the recently developed Kullback-Leibler nearest neighbor (KL-NN) rule approach (*1*) to the problem of automatic classification of stationary electroencephalogram (EEG) time series. In that problem, the category or state of an individual is classified by comparison of his or her EEG with EEG's taken from other individuals. The computation of a Kullback-Leibler (KL) number metric or measure of the difference between two different stationary EEG time series is a key point of our approach. We report here on the automatic classification of anesthesia levels L1 and L3, respectively the anesthesia levels insufficient and sufficient for deep surgery, by machine computations on the EEG alone. Extension of the KL-NN rule approach to distinguish between more than two categories or anesthesia levels does not involve any new concepts.

The anesthesia level EEG data discussed here originated in an experiment at Vancouver General Hospital. In that experiment, 280 epochs of visually screened EEG's that were relatively free of artifact and reflected stationary halothane–nitrous oxide anesthesia level were collected from 20 individuals in surgery. The anesthesia levels, determined by non-EEG criteria, were classified by a single anesthesiologist to eliminate the problem of interrater variability for EEG's. Details of the surgical anesthesia procedures and a review of the status of automatic classification of anesthesia levels by EEG data appear elsewhere (*2*). The data consisted of 64-second recordings of four-channel EEG epoch data (F4-C4, F3-C3, C4-02, and C3-01 in the 10-20 EEG system) analog-frequency modulation recorded through a 0.54- to 30-Hz band-pass filter and then digitally transcribed at 128 samples per second. An examination of the available data suggested that we confine our attention to a two-category classification problem, to classify anesthesia levels L1 and L3, which are, respectively, insufficient and just sufficient for deep surgery. The data selected for analysis were 73 EEG epochs, all the 35 L1 EEG epochs available and 38 L3 EEG epochs (in sets of two to three per individual) from a total of 18 different individuals. The analysis was performed on the first 20-second intervals of each EEG data epoch at a reduced data rate of 128/3 samples per second on $d = 4$ and $d = 2$ EEG data channel data (C4-02 and C3-01).

The implicit conjecture in the EEG population screening problem is that there is sufficient information in the EEG alone to achieve clinically acceptable levels of discrimination between EEG state categories. The credibility of this conjecture is strained by evidence of the broad intersubject EEG variability. The data in Fig. 1, two-channel 20-second anesthesia level L1 and L3 EEG epochs from five subjects, illustrate this broad intersubject EEG variability. The L1 data appear to be relatively homogeneous "fast" EEG's whereas the L3 data include fast, slow regular, and irregular EEG's. The bottom two L3 EEG's (labeled F145, L3, S71 and F170, L3, S73) appear more similar to L1 EEG's than to other L3 tracings. No obvious visual properties distinguish the L1 from L3 EEG's.

A useful statement of the conjecture in the EEG population screening problem is as follows: given labeled EEG samples from two categorical populations, estimate the theoretically best achievable statistical classification performance. The use of a KL-NN classification, in which one subject's EEG is deleted at a time in classification of the labeled EEG sample data base, yields that desired es-

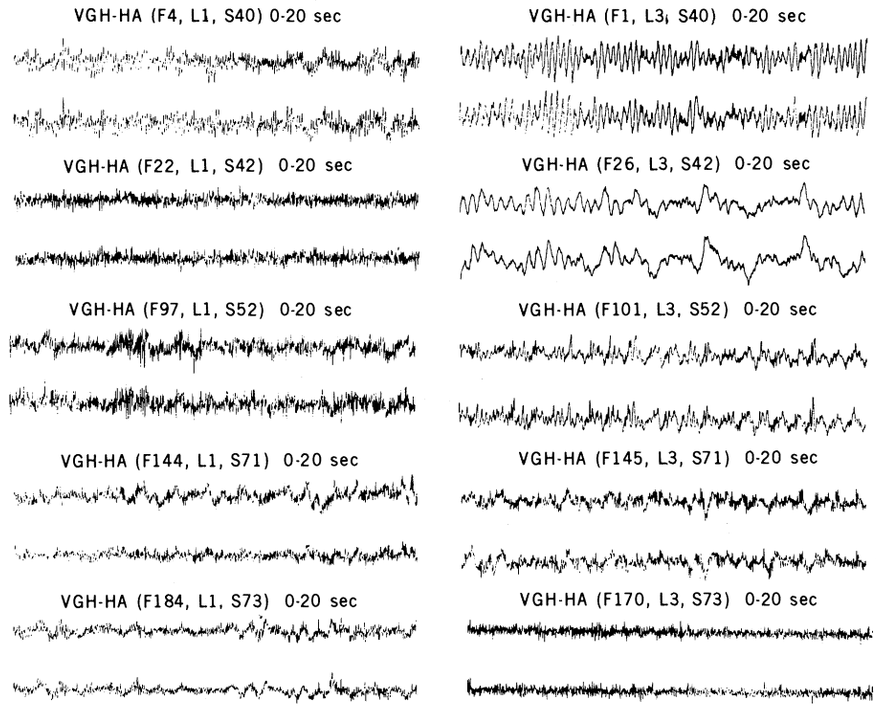

Fig. 1. Twenty-second bipolar (C3-01 or C4-02) epochs of anesthesia levels L1 and L3 from five different individuals.

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timate (*3, 4*). Our specific contributions in the development of this methodology are (i) the demonstration that KL numbers between second-order stationary time series have an optimal minimum probability of error classification; (ii) time and frequency domain formulas for computing the KL numbers; (iii) a demonstration that KL numbers between time series have sufficient metric properties for NN rule classification; (iv) the development of practical computational methods for computing KL numbers; and (v) a worked computational example using clinical EEG population screening classification data that has also been analyzed by previously known techniques [the last is reported here; the others are in (*1*)].

Application of the KL-NN classification rule in the anesthesia level EEG classification problem involves the following steps: (i) Stationary time series EEG samples labeled for anesthesia level L1 or L3 are assumed to be available from different individuals (*5*). (ii) A KL number measure of the difference between a new, to be classified EEG and each of the labeled EEG samples is computed. (iii) The new EEG is classified as is that labeled sample EEG for which the KL number is smallest. In a variation of the KL-NN rule, the KL-*k*NN rule, the new EEG is classified with the label of the majority of its k nearest neighbors (for k an odd integer) (*3, 4*).

A baseline appraisal of the achievable discriminability between the L1 and L3 anesthesia level EEG sample populations was obtained by a cross-validation study (*4, 6*). The EEG epochs of a single individual at a time were deleted from the 18-individual, 73-epoch labeled sample EEG data. Each of the deleted individual's EEG epochs was classified against the remaining 17 individual labeled EEG sample population by the KL-NN and KL-*k*NN rules. The best classification results for the $d = 2$ EEG data channels, achieved with the KL-3NN rule, were one L1 and ten L3 epoch classification errors, with a resulting performance of 97, 74, and 85 percent for the L1, L3, and overall correct classification performance, respectively. The best classification performance for the $d = 4$ EEG data channels, achieved with the KL-NN rule, were one L1 and seven L3 epoch classification errors, with a resulting performance of 97, 82, and 89 percent for the L1, L3, and overall correct classification performance, respectively (*7*). The performance of the KL-NN rules reported here with the anesthesia level EEG data in the EEG populations screening problem is perhaps the best automatic EEG clinical classification ever obtained (*8*).

We implemented the KL number computations by time domain parametric model computations on the EEG time series. That implementation involves the following: Let $\{y^{(j)}(t);\ t = 1, \ldots T;\ j = 0, 1, \ldots n\}$ denote the EEG time series at time t, the index $j = 0$ identifies the new EEG, and $j = 1, \ldots n$ identifies the labeled sample EEG's. (i) Compute the sample $d \times d$ matrix covariance function for each of the $j = 0, 1, \ldots n$ time series. (ii) Fit an autoregressive (AR) parametric time series model to each EEG time series covariance function (*9*). (The covariance function and AR model of each of the labeled sample EEG's are assumed to be stored in the computer.) (iii) Estimate the KL number $I(o, m)$ $(m = 1, \ldots m)$ between the new zero-term EEG and each of the labeled sample EEG's by the formula

$$I(o, m) = \ln \frac{|V_m|}{|V_o|} + \operatorname{tr} \sum_{i=0}^{p_m} \sum_{j=0}^{p_m} A^{(m)}(i)\, C^{(o)}(i - j)\, A^{(m)}(j)' V_m^{-1} - d \quad (1)$$

In Eq. 1, $|A|$, $\operatorname{tr}A$, A', and A^{-1} denote the determinant, trace, transpose, and inverse of the $d \times d$ matrix A, respectively, ln denotes the natural logarithm, $C^{(o)}(\cdot)$ denotes the sample covariance matrix of the new EEG time series, and $\{A^{(m)}(i);\ i = 0, 1, \ldots p_m\}$ denotes the AR model coefficients for the mth labeled EEG. Also, p_m is the automatically determined AR model order and V_j, $j = o$ or m, is the residual variance matrix of the jth EEG time series that is computed during the AR modeling computation (*9*). These computations can be done on minicomputers for research applications or with array processors or chips for real time applications (*10*). Additional considerations for the implementation of KL-NN rules, such as the consequences of alternative EEG normalizations on classification performance and KL-NN cluster analysis considerations to economize on computational and data storage burdens, are treated in (*1*).

A spectral analysis–discriminant analysis method has been dominant for the classification of stationary EEG time series (*11*), and for other applications (*12*). In that method, the EEG time series are first spectrum-analyzed. Spectral features (typically the average power in the sigma, delta, alpha, and beta frequency bands and spectral coherences between pairs of EEG channels in those frequency bands) that are thought to be potentially relevant for discriminating between the alternative classes are abstracted from that analysis for each labeled sample EEG. Those features are analyzed by a stepwise linear, quadratic, or other discriminant analysis to determine the combinations of best discriminating features and to evaluate the corresponding classification performance. The outstanding technical difficulty with that method is that it is ad hoc. The problem for which that solution is optimum is not known. If a comprehensive EEG classification study by all conceivable spectral features and discriminant rules does not yield satisfactory classification performance results, the only technically valid conclusion is that "spectral analysis doesn't work here." No insight is necessarily gained by that experiment on the theoretically smallest probability of error discrimination between the alternative EEG classes. The practical shortcomings of the spectral analysis-discriminant analysis approach to the EEG population screening problem were apparent in the first research with that method (*11*). The spectral features that best discriminated EEG categories in an individual differed for individuals. The loss of discrimination efficiency or equivalently a blurring of features for discrimination with an increase in the number of individuals very likely explains the meager success achieved to date with the classification of clinical EEG's (*11*).

In contrast with the spectral analysis–discriminant analysis method, KL-NN rules are "featureless," and they have definite optimal properties for minimum probability of misclassification (*1, 7*). The KL number is a measure of the dissimilarity between time series; it is not a property of an individual time series, as are spectral features. It is computable by either time domain or frequency formulas (*1*). With only a relatively small number of labeled sample EEG's, the KL number metric used with NN or *k*NN classification rules in a delete-one classification of the labeled EEG data base yields a statistically reliable estimate of the smallest possible probability of EEG misclassification (*3, 4*). Thus the KL-NN rules do yield a test of the implicit conjecture in the EEG population screening problem as well as an implementation to realize the best achievable classification performance.

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References and Notes

1. The KL number measure of the difference between two probability distributions and its properties is in S. Kullback, *Information Theory and Statistics* (Wiley, New York, 1958). A stationary time series is one whose statistical properties do not vary over time. Developments and extensions of the KL measure to problems of discrimination in stationary time series are in W. Gersch, "Discrimination between stationary Gaussian time series, large sample results" (Technical Report No. 30, Project Director, T. W. Anderson, Department of Statistics, Stanford University, 1977); papers presented at annual meeting of The American Statistical Society, 1978, and at Fourth International Joint Conference on Pattern Recognition, Kyoto, Japan, 1978.
2. J. A. McEwan, G. B. Anderson, M. D. Low, L. C. Jenkins, *IEEE Trans. Biomed. Eng.* **22**, 229 (1975); J. A. McEwan, thesis, University of British Columbia (1975).
3. With an increasing number of labeled samples, the probability of misclassification achieved with NN and *k*NN rules is not worse than twice the theoretically smallest probability of misclassification. A delete-one estimate of the probability of misclassification using NN and *k*NN classification rules on a labeled sample data base has an asymptotic bias and mean square error proportional to the reciprocal of the number of labeled samples [T. M. Cover and P. Hart, *IEEE Trans. Inf. Theory* **13**, 21 (1967)].
4. W. H. Rogers, thesis, Stanford University (1976).
5. Automatic parametric time series methods for the selection of stationary time series epochs are described by T. Ozaki and H. Tong [*Proceedings, Eighth Hawaii International Conference on System Sciences* (Western Periodicals, Hollywood, Calif., 1975), p. 224].
6. F. Mosteller and J. W. Tukey, data analysis including statistics, in *Handbook of Social Psychology*, G. Lindzey and E. Aronson, Eds. (Addison-Wesley, Reading, Mass., 1968) vol. 2, pp. 133–160; M. Stone, *J. R. Statist. Soc. Ser. B* (1974), p. 111.
7. The L1 and L3 anesthesia level EEG's are likely not to be equally well classified. The overall probability of classification error is minimized by the KL-NN rules independent of the prior possibilities on costs for different classification errors (*1*). The statistical properties of the KL-NN and KL-*k*NN rules (*3*, *4*) imply that the best estimate of the achievable discrimination performance is obtained by the best performing KL-*k*NN rule.
8. McEwan studied anesthesia level EEG classification using spectral analysis-discriminant analysis methods on the same data base described in the text. Sixty-five and 80 percent correct classification performance on L1 and L3 anesthesia level EEG's was achieved with 13 and 26 prespecified discrimination features, respectively (*2*). McEwan's are probably the best previous EEG clinical population screening results obtained.
9. W. Gersch and J. Yonemoto, *Comput. Biomed. Res.* **10**, 113 (1977); R. H. Jones, *IEEE Trans. Autom. Control* **19**, 894 (1974); W. Gersch, *Math. Biosci.* **7**, 205 (1970).
10. We used a time-shared PDP/1145 computer. For $d = 2$ ($d = 4$) EEG data channels, 853 sample/channel time series, computational steps (i) and (ii) each required approximately 20 (90) seconds. A brute force approach to the computation of step (iii) required 1.2 (4.8) minutes. Our FORTRAN programs for those computations are available upon request. Array processors connected to PDP 11 machines can implement those computations 100 times faster. Such parametric time-domain computations have been implemented for speech recognition research (J. Makhoul, Bolt, Beraneck and Newman, Cambridge, Mass., personal communication). For the $d = 1$ data channel those computations are done in tens of milliseconds. Chips to do steps (i) and (ii) for the $d = 1$ channel exist for real-time secure voice communication (C. S. Miller, C. F. Motley, R. A. Allen, in *EASCON-77*, IEEE catalog No. 77CH1255-9). Extension of the existing array processor implementations for the $d = 1$ channel to process an arbitrary number of channels is straightforward.
11. D. O. Walter, J. M. Rhodes, W. R. Adey, *Electroencephalogr. Clin. Neurophysiol.* **22**, 22 (1967). This was the first major paper to use the spectral analysis-discriminant analysis method. Surveys of that approach to automatic EEG classification are found as follows: A. S. Gevins, C. L. Yeager, S. L. Diamond, J. P. Spire, G. M. Zeitlin, A. H. Gevins, *Proc. IEEE* **63**, 2382 (1975); P. Kellaway and I. Petersen, Eds., *Automation of Clinical Electroencephalography* (Raven, New York, 1973); W. Gersh and J. Yonemoto, *Comput. Biomed. Res.* **10**, 297 (1977).
12. *Proceedings, Workshop on Pattern Recognition Applied to Oil Identification* (catalog No. 76CH124-6C, 1976); *Proceedings of the International Symposium on Computer Aided Seismic Analysis and Discrimination* (IEEE catalog No. 77CH1244-3C, 1977).

13. This work was supported in part by NSF grant 74-09883 and Air Force Office of Scientific Research grant 78-3564 at the University of Hawaii.

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Hyperthermia and Local Anesthetics: Potentiation of Survival of Tumor-Bearing Mice

Abstract. *Lidocaine infusion of a CA755 mammary adenocarcinoma growing in the hind leg of BDF$_1$ mice results in a significant increase in the animals' survival when combined with heating for 1 hour in a 43.5°C water bath. This ability of local anesthetics to prolong survival following hyperthermia is consistent with the hypothesis that increases in membrane fluidity influence sensitivity to heat. In view of the extensive clinical experience with local anesthetics, the delay between clinical application and the observation that they potentiate the action of hyperthermia in animals may be reduced.*

This report describes the potentiation by an anesthetic of the tumor-inhibiting effect of local hyperthermia. The combined therapy resulted in apparent local cures in four of 31 animals.

In studies aimed at reevaluating the "lipoid liberation" theory of Heilbrunn (*1*), we obtained evidence in support of the hypothesis that the fluidity of membranes as determined by their lipid content is a major contributing factor in the death of cells exposed to hyperthermia. Singer (*2*) observed that a lower temperature was required for an increase in liposome permeability (^{22}Na efflux) when local anesthetics were present. Local anesthetics increase marker molecule mobility and fluidity in intact cell membranes and cell membrane fractions and increase the rotation of probe molecules dissolved in synthetic protein-free lipid bilayers (*3*). We have shown (*4*) that procaine-HCl increases hyperthermic killing of an unsaturated fatty acid auxotroph of *Escherichia coli*. Data obtained with V79 Chinese hamster lung cells (*5*) also show a potentiation of hyperthermic killing by procaine. On the basis of these observations, we postulated that local anesthetics might potentiate the therapeutic effect of hyperthermia in treatment of malignant disease (*4*).

Young adult BDF$_1$ mice grafted with mammary adenocarcinoma strain CA755 were used throughout. For transplantation, tumors were removed from donor mice and a crude suspension was prepared with the aid of a Snell cytosieve (*6*). When the tumors measured approximately 4 mm in mean diameter, the mice were randomly assigned to treatment groups. Tumor sizes were determined by caliper measurement of the maximum dimension. These data will be reported separately (*7*). Responses vary from disappearance of tumors to delays in growth to a reduced rate of growth after treatment.

Mice were anesthetized by intraperitoneal injection of 14 mg of chloral hydrate. They were placed on special carriers with the tumor-bearing leg drawn through an opening for immersion in a Tecam constant-temperature bath with a TU Tempunit circulating heater and a Yellow Springs Instrument telethermometer thermistor probe. The legs of the mice were gently held in place by masking tape over the lower portion of the limb during heating. The tumor-bearing legs were exposed to bath temperatures of 42° or 43.5°C (± 0.1°C) for 1 hour. Water surfaces were insulated with plastic spheres 2 cm in diameter both to aid in maintaining constant bath temperature and to further insulate the remainder of the animals' bodies from heat. The air temperature above the water bath at the level of the mouse carrier did not exceed 37°C.

Lidocaine-HCl (Elkins-Sinn, Inc.) was infused by injection into three areas of the tumor in a volume of 0.05 ml (2 mg per mouse) within 5 minutes before heat treatment. Some comparison groups were similarly infused with 0.05 ml of isotonic saline. In each experiment, nine groups of animals were randomly assigned to a 3 by 3 block design of treatment regimes with three temperatures [room temperature (22° ± 1°C), 42°, and 43.5°C] (*8*), and three injection options (no injection, saline injection, and lidocaine injection).

There was a significant interaction between lidocaine infusion and the temperature of heating in all three experiments thus far completed. Mean survival of the mice with lidocaine-infused tumors heated at 43.5°C for 1 hour was signifi-

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