creases in size during adult life (*13*), and the percentage increase in the size of the granule cell layer from 3 to 24 months is similar in magnitude to that predicted by the percentage of heavily labeled granule cells at 3 months (see above).

Thus, we conclude that the labeled granule cells observed in dentate gyrus and olfactory bulb of the adult rat represent newly formed neurons. A corollary of this conclusion is that the synapses found on labeled granule cells in the olfactory bulb must also have been newly formed in an adult animal.

These results indicate that the old concept that the adult mammalian brain is largely static is no longer tenable. Numerous studies have shown that experimental manipulations can lead to growth and plasticity in adult brain (*18*). Now we have confirmed that growth and plasticity, including neurogenesis and synaptogenesis, can also occur in the mature, unoperated, mammalian brain.

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References and Notes

1. J. B. Angevine, Jr., *Exp. Neurol.* **13** (suppl. 2), 1 (1965); B. Schultze, B. Nowak, W. Maurer, *J. Comp. Neurol.* **158**, 207 (1974); A. R. Schlessinger, W. M. Cowan, D. I. Gottlieb, *ibid.* **159**, 149 (1975).
2. J. W. Hinds, *J. Comp. Neurol.* **134**, 287 (1968).
3. J. Altman and G. D. Das, *ibid.* **124**, 319 (1965); J. Altman, *Nature (London)* **207**, 953 (1965); _____ and G. D. Das, *J. Comp. Neurol.* **126**, 337 (1966); S. A. Bayer, *Neurosci. Abstr.* **6**, 365 (1976).
4. J. Altman, *J. Comp. Neurol.* **137**, 433 (1969).
5. _____, in *The Neurosciences. A Study Program*, G. C. Quarton, T. Melnechuck, F. O. Schmitt, Eds. (Rockefeller Univ. Press, New York, 1967), p. 723.
6. _____, *Anat. Rec.* **145**, 573 (1963).
7. C. Perachia and B. S. Mittler, *J. Cell Biol.* **53**, 234 (1972).
8. W. M. Cowan, D. I. Gottlieb, A. E. Hendrickson, J. L. Price, T. A. Woolsey, *Brain Res.* **37**, 21 (1972).
9. J. W. Hinds and P. L. Hinds, *J. Neurocytol.* **1**, 169 (1972); D. W. Vaughan and A. Peters, *J. Comp. Neurol.* **149**, 355 (1973).
10. A. Peters, S. L. Palay, H. deF. Webster, *The Fine Structure of the Nervous System* (Saunders, Philadelphia, 1976).
11. J. L. Price and T. P. S. Powell, *J. Cell Sci.* **7**, 125 (1970).
12. The number of heavily labeled granule cells observed was 0.0307 percent of the total granule cell population (TGP) examined at 3 months or 0.000307 × TGP. The length of the S phase of subependymal cells [the probable source for late arising granule cells in the olfactory bulb (*4*)] in the adult rat has been estimated at 8.5 hours [P. D. Lewis, *Exp. Neurol.* **20**, 203 (1968)]. If we assume that the tritiated thymidine is available for incorporation into DNA for only a short time, less than an hour, 8.5 hours after injection almost all the labeled cells would have left S phase and another equally large population of cells could be labeled if another injection was given. This would then lead to a doubling of the number of heavily labeled granule cells seen in the olfactory bulb 30 days after injection compared with a single injection. On the assumption that neuron production continues at the same rate from 3 months (90 days) to 24 months (730 days), if one injected every 8.5 hours, the total number of heavily labeled cells that would be seen would be 640 days ÷ 8.5 hours = 1807 times the number of heavily labeled cells actually observed after the single injection at 3 months. The percentage increase in the number of granule cells from 3 to 24 months is therefore (0.000307)(TGP)(1807)(100)/TGP = 55 percent.
13. J. W. Hinds and N. McNelly, *J. Comp. Neurol.* **171**, 345 (1977).
14. M. C. Diamond, R. E. Johnson, C. A. Ingham, *Behav. Biol.* **14**, 163 (1975).
15. V. Marěs, B. Schultze, W. Maurer, *J. Cell Biol.* **63**, 665 (1974).
16. R. L. Sidman, in *Contemporary Research Methods in Neuroanatomy*, W. J. H. Nauta and S. O. E. Ebbeson, Eds. (Springer, Berlin, 1970), p. 252.
17. S. Fujita, S. Yoshida, M. Fukuda, *Acta Histochem. Cytochem.* **4**, 126 (1971); S. Fujita, *J. Comp. Neurol.* **155**, 195 (1974).
18. J. J. Bernstein and D. C. Goodman, Eds., *Brain Behav. Evol.* **8**, 1 (1973); L. Guth, *Exp. Neurol.* **45**, 606 (1974); G. Lynch and C. W. Cotman, in *The Hippocampus*, R. L. Isaacson and K. H. Pribram, Eds. (Plenum, New York, 1975), p. 123.
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An Adenylate Cyclase of Brain Reflects Propensity for Breast Cancer in Mice

Abstract. *High propensity for breast cancer in mice was associated with low dopamine-stimulated adenylate cyclase activity in the brain, low spontaneous motorization, and low motor responses to injections of the catecholamine precursor,* L-*dopa.*

Previous experiments showed that an increase occurred in the mean life span of mice consuming L-dopa (L-3,4-dihydroxyphenylanine) (*1*). This was ascribed to reduction of intervening diseases, for reasons which included the increased life span in a nonlethal affliction, Parkinson's disease, by L-dopa (*2*).

In Parkinsonism, improvement results from the following sequence. L-Dopa produces dopamine which stimulates an adenylate cyclase in postsynaptic neurons at dopaminergic synapses in the brain (*3*). This adenylate cyclase, discovered by Greengard *et al.* (*4*), is an important part of the dopamine receptor because it produces the adenosine 3′,5-monophosphate (cyclic AMP) which determines the responses of the postsynaptic neuron to dopamine.

Our initial studies and those of others (*5–7*) emphasized that this brain enzyme has behavioral roles because: (i) the cyclase was measured in the caudate nucleus which regulates motor behavior; (ii) it was modified by anti-Parkinson (*8*) and antipsychosis drugs (*9*) in vitro; and (iii) drugs which are normally used to change immunity, carcinogenesis, or protein synthesis have produced in intact mice quantitative correlations between the activity of the cyclase and the behavioral parameters studied earlier (*5, 6*). Nonetheless, a nonbehavioral role is suggested for this enzyme by (i) the cyclase extends beyond the caudate nucleus (*10, 11*) into areas which perhaps include the hypothalamus, a structure controlling hormonal function and metabolism, and (ii) feeding L-dopa to mice in amounts which usually increase life span potentiates the dopamine-stimulated cyclase (*12*). We suspected, therefore, that this enzyme might be a part of a system of the brain which registers or regulates the incidence of intervening diseases. To examine this hypothesis, we tested the cyclase in the brains of mice treated with immunotherapeutic materials and in the brains of untreated animals having a known propensity for a major disease, cancer.

The immunotherapeutic agents were given to groups of 36 female C3H/HeJ mice, 5 to 6 weeks old. One subgroup of 18 mice was injected intraperitoneally with BCG vaccine (1.5 mg in 0.5 ml of normal saline) (*13*) and another subgroup of 18 mice with normal saline solution once a week for 3 weeks. Similar subgroups were injected either with *Corynebacterium parvum* vaccine (1.4 mg in 0.2 ml of saline) (*14*) or with normal saline solution once a week for 3 weeks. The net dopamine-stimulated activities of the adenylate cyclase were measured after the addition of 200 μM of dopamine hydrochloride to the homogenates of the caudate nuclei as described (*8*). These activities, computed as picomoles of cyclic AMP per combined caudates of each animal were as follows: (i) BCG injected, 112 ± 14.8; controls, 59 ± 10.8 ($P < .01$); (ii) *C. parvum* injected, 122.5 ± 4.4; controls, 54 ± 10 ($P < .01$). The experiments with the *C. parvum* vaccine were repeated in their entirety on CD-1 male mice (5 to 6 weeks old) and C3H/HeJ breeder female mice (7 months old) with similar results.

In parallel experiments, mice were vaccinated with BCG or *C. parvum* and tested for their motor responses to intraperitoneally injected L-dopa (*6*). These

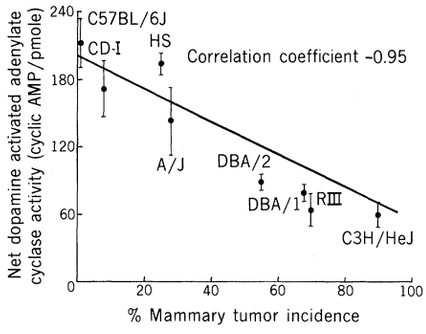

Fig. 1. Correlation between the mean net dopamine- (200 μM) stimulated adenylate cyclase activities of female mice from the strains indicated and the incidence for breast cancer exhibited by these strains in studies by others. Only the incidence of subcutaneous cancer (not of breast cancer) had been calculated in the HS mice (*16*). The incidence of breast cancer is thus exaggerated.

showed marked motor hyperreactivity compared to their nonvaccinated controls.

Earlier, agents which can enhance some aspect of immunity had increased the net dopamine-stimulated cyclase activity and vice versa in intact mice (*5, 6*). The results of the vaccinations with BCG and *C. parvum* strengthened this observation. We noted, furthermore, that the C57BL/6J females, which have a low propensity for breast cancer, showed higher enzymatic activities than did the C3H/HeJ females, which have a high incidence of the disease. These considerations raised the possibility that the activity of the cyclase might be predictive of the incidence of spontaneous breast cancer. We therefore tested groups of 6- to 7-month-old female breeder mice without evident cancer, from eight strains whose incidence of breast cancer had been published by others (*15, 16*). Each group consisted of 30 mice, at least 12 of which were used for measuring the basal, the total, and the net dopamine-stimulated adenylate cyclase activities in homogenates of caudate nuclei (*6, 8, 12*). The rest were tested for their behavioral responses to injected L-dopa. These experiments were performed randomly, whenever mice were received.

A striking correlation (Fig. 1) emerged after the mean net dopamine-stimulated adenylate cyclase activities for each strain were plotted against the percentages of animals expected to develop breast cancer.

The Pearson correlation coefficient between the mean of the dopamine-stimulated adenylate cyclase activities and the percentage of animals in each strain expected to develop breast cancer was $-.95$ ($P < .0001$) (Fig. 1) and the correlation coefficient between the mean of the logarithm of the specified enzyme activity and the percentage of mammary tumor incidence expected was -0.97 ($P < .0001$). The Spearman rank correlation was $-.98$ ($P < .01$). The Pearson correlation coefficient calculated from all the individual measurements instead of the means was $-.85$, and calculated from the logarithm of the enzymatic measurements to stabilize the variances the coefficient was $-.89$ ($P < .001$). When the sum of the dopamine-stimulated and the basal adenylate cyclase activity for each of these eight strains of mice was plotted instead, the correlation coefficient was $-.94$. In contrast, when only the basal activity, which was not significantly different from one strain to the other, was considered, no line could be obtained (correlation coefficient, .095). This is consistent with the finding of Mishra *et al.* (*7*) who claimed that the major portion of the basal adenylate cyclase activity of caudate is anatomically distinct from the postsynaptic dopamine-sensitive cyclase system.

In these experiments, absence of cancer was determined only by inspection, palpation, and dissection. To determine possible effects on the cyclase from actual presence of cancer an experiment was performed on C3H/HeJ breeder mice with fully developed cancer of the breast. Controls were similar mice without palpable or visible cancers. The mean net dopamine-stimulated adenylate cyclase activity in the cancer-bearing animals was 54.7 ± 16.5 pmole of cyclic AMP per combined caudates of each animal versus 63.5 ± 8.9 in the control group, a nonsignificant difference. Thus, fully developed cancer either had not affected the brain enzyme or had caused it to become fixed. To test this alternative we administered L-dopa (40 mg per gram of Purina chow) for 3 months to one group of mice with gross tumors and to another group without visible tumors. The mean activity of the cerebral cyclase in the tumor-bearing animals was 53 ± 10.5 pmole of cyclic AMP whereas that of the "tumor-free" mice was 108.7 ± 19.7 ($P < .05$). This argued that fully developed breast cancer was rendering the activity of the adenylate cyclase in the brain nonresponsive to L-dopa.

Changing the activity of the cyclase of the caudates with drugs has thus far always changed correspondingly the reactions of intact mice to injected L-dopa (*5, 6*). This made it interesting to know whether the untreated animals shown in Fig. 1 would rank themselves the same

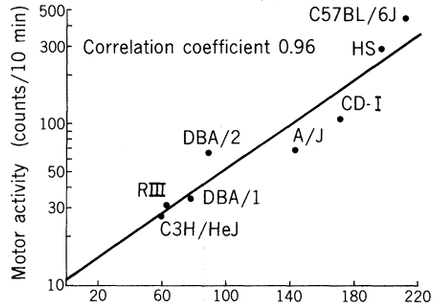

Fig. 2. Correlation between the mean net dopamine- (200 μM) stimulated adenylate cyclase activities of female mice from the strains indicated and the mean of the logarithm of the motor activity determined in an Animex automatic activity meter for 10 minutes, 40 minutes after intraperitoneal injection of L-dopa (1.2 mg per gram of body weight). Some of the most responsive animals (C57BL/6J) died an hour after the activity measurements were completed.

way if tested for motor reactions induced by single intraperitoneal injections of L-dopa (1.2 mg per gram of body weight). This experiment was particularly germane since the C3H/HeJ mice which have a high propensity for cancer were normally much more inactive than the C57BL/6J whose propensity for cancer is low. In the next experiments, six groups of three mice from each of the eight strains were injected with L-dopa and placed on an Animex automatic motor activity meter. The means of the ensuing measurements, when plotted against the expected incidence of cancer on a semilogarithmic scale, appeared approximately linear with a coefficient of correlation of $-.87$ ($P < .01$). The Pearson correlation coefficient between the mean of the logarithm of the motor activity measurements and the mean of the enzymatic activities was .96 ($P < .001$) (Fig. 2). These measurements strongly suggested that the behavioral function of the cyclase was linked to its ability to predict propensity for breast cancer.

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References and Notes

1. G. C. Cotzias, S. T. Miller, A. R. Nicholson, Jr., W. H. Maston, L. C. Tang, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 2466 (1974); G. C. Cotzias, S. T. Miller, L. C. Tang, P. S. Papavasiliou, Y. Y. Wang, *Science* **196**, 549 (1977).
2. M. M. Hoehn and M. D. Yahr, *Neurology* **17**, 427 (1967); R. D. Sweet and F. H. McDowell, *Ann. Intern. Med.* **83**, 456 (1975); F. G. Diamond and C. H. Markham, *J. Neural Transm.* **38**, 259 (1976).
3. A. Carlsson, M. Lindqvist, T. Magnusson, *Na-*

ture (London) **180**, 1200 (1957); G. C. Cotzias, P. S. Papavasiliou, J. Z. Ginos, A. Steck, S. E. Düby, *Annu. Rev. Med.* **22**, 305 (1971).
4. P. Greengard, J. A. Nathanson, J. W. Kebabian, in *Frontiers in Catecholamine Research*, E. Usdin and S. Snyder, Eds. (Pergamon, New York, 1973), p. 377; J. W. Kebabian, G. L. Petzold, P. Greengard, *Proc. Natl. Acad. Sci. U.S.A.* **69**, 2145 (1972).
5. L. C. Tang, G. C. Cotzias, M. Dunn, *Proc. Natl. Acad. Sci. U.S.A.* **71**, 3350 (1974).
→ L. C. Tang and G. C. Cotzias, *ibid.* **74**, 1242 (1977).
→ R. K. Mishra, E. L. Gardner, R. Katzman, M. H. Makman, *ibid.* **71**, 3883 (1974).
→ L. C. Tang and G. C. Cotzias, *ibid.* **74**, 769 (1977).
→ Y. C. Clement-Cormier, J. W. Kebabian. G. L. Petzold, P. Greengard, *ibid.* **71**, 1113 (19 → M. Karobath and H. Leitich, *ibid.*, p. 2915; P. Seeman and T. Lee, *Science* **188**, 1217 (1975).
→ A. S. Horn, A. C. Cuello, R. J. Miller, *J. Neurochem.* **22**, 265 (1974); J. W. Kebabian and J. M. Saavedra, *Science* **193**, 683 (1976).
11. K. Gale, A. Guidotti, E. Costa, *Science* **195**, 503 (1977).
12. L. C. Tang and G. C. Cotzias, *Proc. Natl. Acad. Sci. U.S.A.* **74**, 2126 (1977).
13. D. W. Weiss, R. S. Bonhag, K. B. DeOme, *Nature (London)* **190**, 4779 (1961).
14. J. E. Castro, *Eur. J. Cancer* **10**, 121 (1974).
15. S. Nandi and C. M. McGrath, *Adv. Cancer Res.* **17**, 353 (1973); W. G. Hoag, *Ann. N.Y. Acad. Sci.* **108**, 805 (1963).
→ H. A. Johnson and E. P. Cronkite, *Radiat. Res.* **30**, 488 (1967).
17. Supported by NIH grant NS 12776, the late Mrs. Jean Mauze, the American Parkinson's Disease Association, the Charles E. Merrill Trust, and Mrs. Katherine Rodgers Denckla. Y. Y. Wang of the Biostatistics Department, Memorial Sloan-Kettering Cancer Center, conducted the statistical evaluations. D. J. Donahue obtained all primary data on motor activity.
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Communication Deviance in the Families of Schizophrenics: A Comment on the Misuse of Analysis of Covariance

Abstract. *Serious contradictions in recent research programs concerning communication anomalies in parents of schizophrenics have been generated by invalid statistical analyses. The method of analysis used, the analysis of covariance, can lead to erroneous conclusions in the context of these studies, and thus, other means must be sought for bringing these important research programs into common focus.*

A growing body of research is devoted to establishing the existence and etiological significance of abnormal styles of communication in the families of schizophrenics (*1–3*). A hypothesis underlying much of this research is that when speech anomalies such as vagueness, irrelevance, and lack of closure characterize ongoing parental transactions, the likelihood of schizophrenia in the offspring is greatly increased. These communication difficulties are believed to create a poor sense of reality and a difficulty in modeling patterns of clear and logical thinking so that when the offspring is faced with life stress, more primitive cognitive mechanisms are likely to appear. Thus, it is hypothesized that deviant parental communication styles lay the groundwork for the subsequent appearance of the core symptoms of a schizophrenic psychosis.

It is generally acknowledged that conclusive testing of the etiological aspects of the hypothesis must await the completion of longitudinal studies that can determine whether parental communication deviance precedes, and therefore is not reactive to, the onset of schizophrenia in the offspring (*4*). Because such prospective studies are difficult to carry out and take many years to complete, early studies have been based upon the more readily accomplished (though weaker) cross-sectional strategy in which the communication deviance of parents of schizophrenics is contrasted with that observed in parents of severely disturbed, nonschizophrenic offspring (*5*). The initial series of these cross-sectional studies carried out by Wynne and Singer and their colleagues (*1*) have provided support for the communication deviance hypothesis and have lent great importance to the conduct of the etiologically more informative longitudinal research programs.

Communication deviance, as employed in these studies, is measured by counting the number of units of abnormal verbal behavior that fit preestablished categories. Typically used are projective test data, in which there is wide variability in the number of verbal units of all kinds observed; the question has been raised as to whether the communication deviance index is simply an artifact of the number of words spoken. Hirsch and Leff (*2*, *3*), in a carefully conducted replication of the Wynne-Singer procedures with an English sample of parents of schizophrenics and parents of neurotics, found group differences in communication deviance in the expected direction but with considerable group overlap. These investigators then raised the question of whether these group differences in communication deviance are, in fact, artifacts of verbosity differences between the groups in their sample. The question posed by Hirsch and Leff was, "If both groups had spoken the same mean number of words, would we expect there to be any difference between their mean deviance scores?" (*2*, p. 144). Analysis of covariance, a statistical technique for estimating group differences with the effects of a correlated variable removed, was applied to these data. The previously found significant differences between groups in communication deviance disappeared, leading Hirsch and Leff to conclude that they had disconfirmed the Wynne and Singer findings.

Wynne and Singer and their colleagues also applied the analysis of covariance to their own data, but found that differences in parental communication deviance did *not* vanish but were slightly accentuated when verbosity was employed as a covariate (*1*, p. 43). Thus, a major source of disagreement between these studies has been generated by attempts to use the analysis of covariance to control statistically for group differences in verbosity.

We will demonstrate that the analysis of covariance is not applicable in these cross-sectional studies, even though the traditional analysis of covariance assumptions of linearity and equality of within-group regression coefficients were met. Although the problems involved are subtle, it is nevertheless important that the analysis of covariance be avoided in research studies in which the technique can lead to erroneous conclusions.

The inapplicability of the analysis of covariance in these research programs stems from two problems. First, the groups (for example, the parents of schizophrenics and the parents of disturbed nonschizophrenics) are not created through random assignment. Under these circumstances, observed group differences in verbosity (the covariate) may arise from complex effects of selection, from genetic differences among parents, or from the illnesses of the offspring. In fact, the same factors that are responsible for differences in communication deviance (the dependent measure) may be responsible for differences in verbosity.

Second, the "true" relationship between verbosity and communication deviance cannot be known for a given sample of individuals. Except in the extreme where only a few words are spoken, this relationship could vary as a function of innumerable situational factors such as the nature of the stimulus cards, the instructions, and the recent experiences of subjects. These multiple, possibly interacting sources of measurement error can be given explicit recognition, as in the theory of generalizability where reliability is conceptualized and estimated in a multifaceted framework (*6*). In the studies under discussion, the