diminishes in amplitude and usually reverses its polarity; the ERS, by contrast, increases in amplitude with penetration into the cortex to approximately the inversion point of the ER, then diminishes to disappear at a depth where the electrode presumably penetrates white matter. Third, either warming or cooling the oil bath (± 5°C) away from 37°C has little effect upon the amplitude of the ER, but strikingly reduces the ERS amplitude, as Fig. 2 illustrates. Such dissociations between the two phenomena can also be demonstrated with manipulations of anesthetic depth and type, trauma, and asphyxia; for example, in recordings made on animals near death the ERS may disappear while the ER is still clearly present. When an animal finally stops breathing, the baseline resistance of its brain rises over a period of 20 to 30 minutes to reach final values around 6000 (versus 2000 at 1 khz), 5000 (versus 1500 at 10 khz), and 4000 (versus 1300 at 100 khz) ohms.

Mechanisms postulated for the evoked resistance shift must account for its two phases, an abrupt decrease followed by a more prolonged rise. The normal coincidence of ERS and ER (Fig. 1) suggests that events associated with depolarization of cortical neurons following the sensory input from the thalamus are critical. The simplest explanation of the ERS might therefore hold that reductions in membrane resistivity associated with depolarization of cortical neurons lie at the basis of the phenomenon. This argument is supported by the fact that the ERS reaches its maximum in the region of the pyramidal cell bodies where extensive neuronal membrane depolarizations might be expected. Such an explanation might serve the resistance decrease but no reported measurements show a resistance rise connected with neuronal membrane depolarization or recovery; an additional hypothesis would therefore be required to explain the later phase of increasing resistance in the ERS.

The dissociation between ER and ERS caused by temperature, trauma, and anesthesia, however, argues against a simple neuron depolarization theory, since the ERS may disappear when evidence of unchanged depolarization (a stable ER) exists. Hence other possibilities should be considered. Alterations in the extracellular space through which the measuring current flows is an outstanding possibility. Theoretically the measured resistance of brain would decrease if the extracellular space were to increase in volume or in ionic concentration. Evidence exists that such alterations in volume and composition do take place, though their time course as thus far measured is long compared to the events in the ERS. The diminution of ERS magnitude with increasing bridge frequency is not inconsistent with this possibility, since membrane capacitance would tend to shunt the extracellular path at higher frequencies, and only lower frequencies would detect changes in the extracellular fluid. Less likely possibilities involve changes of membrane resistance in cells other than those engaged in producing the ER. These would include neurons not discharging and glial cells. Synaptically depolarized or hyperpolarized neurons show conductivity increases (*3*); the ERS might depend upon such events in dendritic membrane (which may be more sensitive to anoxia and temperature change than the soma). Glial cells also alter their conductivity when stimulated, showing an initial increase followed by a decrease (*4*), but with a time course many times longer than required to explain the ERS described here for cats.

The remaining current paths that might alter cortical conductivity flow through intracellular fluids, and the blood vessels with their contents. It is unlikely that the ERS would depend upon changes in these factors.

The mechanism or mechanisms responsible for the ERS cannot be determined from the experimental evidence at hand. Neither the early resistance drop nor the later resistance increase can convincingly be assigned to neuronal events responsible for the elaboration of the sensory evoked response.

KENNETH A. KLIVINGTON
ROBERT GALAMBOS
Department of Engineering and Applied Science and *Department of Psychology, Yale University, New Haven, Connecticut 06510*

References and Notes

1. K. S. Cole and H. J. Curtis, *J. Gen. Physiol.* **22**, 649 (1939).
2. A. A. P. Leao and H. M. Ferreira, *Anais Acad. Brasil. Cienç.* **25**, 259 (1953); W. H. Freygang, Jr., and W. M. Landau, *J. Cellular Comp. Physiol.* **45**, 377 (1955); W. R. Adey, R. T. Kado, J. Didio, *Exp. Neurol.* **5**, 47 (1962); W. R. Adey, R. T. Kado, D. O. Walter, *ibid.* **11**, 190 (1965); J. B. Ranck, Jr., *ibid.* **7**, 144 (1963); A. Van Harreveld, T. Murphy, K. W. Nobel, *Am. J. Physiol.* **205**, 203 (1963).
3. J. C. Eccles, *The Physiology of Nerve Cells* (Johns Hopkins Press, Baltimore, 1957).
4. F. D. Walker and T. Takenaka, *Exp. Neurol.* **11**, 277 (1965); W. Hild and I. Tasaki, *J. Neurophysiol.* **25**, 277 (1962).
5. Supported by NIH grant GM 1106 and NSF grant G 23584.

14 April 1967

Aminoacidemias: Effects on Maze Performance and Cerebral Serotonin

Abstract. *The feeding of high dietary supplements of* L-*phenylalanine (7 percent) and* L-*leucine (7 percent) to weanling rats is associated with poor performance in a multiple-T, water-escape maze. Supplements high in* L-*tryptophan (5 percent), on the other hand, result in maze performance which is superior to that of controls. Adding 5 percent tryptophan to the high-phenylalanine diet reverses the behavioral deficit. The quality of maze performance correlated with the cerebral content of serotonin.*

Considerable evidence has accumulated to demonstrate the impairment of maze performance subsequent to the administration of excessive dietary phenylalanine (*1–6*). We have found weanling rats on phenylketogenic diets to be less proficient than controls in mastering a multiple-T water maze (*2*). The water-escape reinforcement precluded the need for water- or food-deprivation which would have introduced additional uncontrolled variables. We also reported preliminarily on the effectiveness of tryptophan in improving performance (*2*). The behavioral deficit induced by phenylketogenic diets was confirmed by Polidora and his coworkers (*3–5*), and malnutrition was eliminated as a critical variable by pair-feeding techniques (*4*).

Behavioral effects have been ascribed to other amino acids when present in high concentrations in blood. Leucine has been associated with a severe degree of mental retardation in humans (*6*), and reversible changes in behavior have been noted in normal subjects following oral administration of high levels of L-tryptophan (*7*). Phenylalanine, leucine, and tryptophan have pronounced effects also on cerebral indole metabolism. Phenylalanine (*1*) and leu-

Table 1. Effect of amino acid feeding on maze performance. There were ten rats in each diet group, and each rat received six trials. S.E., standard error of the mean.

Diet	Mean No. of errors on trial No.:						Mean No. of total errors	S.E.
	1	2	3	4	5	6		
B alone	2.9	4.8	3.0	2.5	2.0	1.6	16.8	2.0
B + Phe	3.7	7.1	5.4	5.3	5.8	4.3	31.6*	3.7
B + Leu	6.2	5.4	5.1	4.4	4.1	3.9	29.1*	4.5
B + Try	1.7	4.2	2.2	1.3	1.1	1.3	11.8*	0.9
B + Phe + Try	2.9	3.8	1.3	2.5	1.1	2.3	13.9†	1.7

* Significantly different from control (Student's t-test), $P < .05$. † Significantly lower than B + Phe rats, $P < .01$

cine (*8*) both reduce serotonin levels in brain, whereas tryptophan (*9*) increases the cerebral content of this amine.

The effects of high amino acid concentrations on maze performance and levels of cerebral serotonin were studied in male Sprague-Dawley rats (4 weeks of age and matched for initial weight) fed the following diets:

B alone = vitamin B-complex test diet, complete (Nutritional Biochemical Corp.), without any supplementary amino acid;

B + Phe = test diet plus 7 percent L-phenylalanine;

B + Leu = test diet plus 7 percent L-leucine;

B + Try = test diet plus 5 percent L-tryptophan;

B + Phe + Try = test diet plus 7 percent L-phenylalanine plus 5 percent L-tryptophan.

The animals were maintained on these diets for 2 weeks, which produced at least a tenfold elevation of the supplementary amino acid in blood. The animals were divided into the five experimental groups noted above, each of which consisted of ten rats. They were allowed to consume their specific diets between runs so that they were never in a fasted condition. Beyond the initial conditioning in a straightaway water channel, two preliminary runs were conducted in the water maze with sliding doors preventing retrogression. Thereafter, the order of testing rats in the maze was randomized for each successive trial until six additional trials had been completed. After each trial, error scores were recorded along with the elapsed time. Errors were scored as the number of times each animal responded incorrectly at the six choice points in the maze. At least 15 minutes separated each trial from the previous one and served to minimize the factor of physical fatigue. The water maze and general procedure followed were essentially those of Biel (*10*). Student's t-test was used to estimate significance of the difference between the means of total errors. After the final trial each animal was killed and the level of cerebral serotonin was determined by the method of Bogdanski (*11*).

Both the phenylalanine- and leucine-treated animals negotiated the maze with significantly more errors ($P < .05$) than the control animals (Table 1). These animals also had significantly slower transit times. Polidora (*4*), who noted a similar behavioral deficit in rats administered 5 to 7 percent dietary L-phenylalanine, concluded that the slower speed of the experimentals is "a direct result of the time-consuming and fatigue-inducing characteristic of errors" and *not* attributable to general debilitation, poor swimming ability, or unusual susceptibility to fatigue.

Unexpectedly, rats on the B + Try diet were superior in performance to all other animals ($P < .01$), including the controls (Table 1). Furthermore, on the B + Phe + Try diet the rats also performed at least as well as the controls. It will be noted that without pair-feeding a weight discrepancy developed between the experimental and control rats (Table 2). All of the animals which were on diets high in amino acids had weights at the time of behavioral testing which were significantly below those of the control animals. The fact that pair-fed rats showed similar results (*4*), and the fact that low-weight animals with tryptophan excess performed well, while low-weight rats with leucine or phenylalanine excesses performed poorly, indicate that the amino acid imbalance is the primary factor mediating differences in maze performance.

Table 2. Effect of high amino acid feedings on weight.

Diet	Final weight	Control (%)
B alone	159.5	100
B + Phe	126.1*	79
B + Leu	108.5*	68
B + Try	103.7*	65
B + Phe + Try	98.9*	62

* $P < .05$.

Cerebral serotonin was significantly below control levels in rats fed the B + Phe and B + Leu diets but was significantly higher than the control in those fed large quantities of tryptophan with or without phenylalanine (B + Try or B + Phe + Try) (Table 3).

The results clearly demonstrate that high concentrations of the various amino acids had differing behavioral and pharmacological effects upon the rat. This was particularly evident with tryptophan, which was capable of reversing the deficit in maze performance produced by phenylalanine. Nonspecific stimulation of spontaneous activity, as measured by means of a vertically revolving activity drum (*12*), was not appreciable in the high-tryptophan animals.

The defect in 5-hydroxyindole metabolism in phenylketonuria (*13*) and in experimental phenylalaninemia (*1, 2*) and leucinemia (*8*) has been well documented. Whether this defect is of primary etiologic significance to the behavioral deficit or an incidental response to the general disruption in cerebral amino acid transport is not clear. The addition of 5 percent tryptophan to the B + Phe diet, which more than corrects the serotonin deficiency, failed to alter significantly the disrupted pattern of cerebral amino acids found in column chromatographic analyses of "phenylketonuric" brains (*14*).

Similarly, the threefold increase in cerebral dopamine (with no appreciable rise in norepinephrine) which Green *et al.* (*9*) found after dietary administration of 18 percent DL-phenylalanine, could not be normalized by the addition of 2 percent DL-tryptophan, although this supplement succeeded in correcting the 32 percent decrease in brain serotonin.

In general, our data support the previous findings of Yuwiler and Louttit, which show a decreased level of cerebral serotonin associated with poor maze performance in experimental "phenylketonuria" (*1*). These authors, based on their finding that rats with *elevated* brain serotonin induced by a monoamine oxidase inhibitor also displayed impaired maze performance, concluded that the decrease in serotonin following excessive phenylalanine may be an

Table 3. Effect of high amino acid feedings on brain serotonin concentration, after the five groups of rats had been on their particular diets for 14 days.

Diet	Brain serotonin (mμg/g)	Control (%)
B alone	485	100
B + Phe	430 ($P < .05$)	89
B + Leu	420 ($P < .05$)	87
B + Try	685 ($P < .05$)	141
B + Phe + Try	550 ($P < .05$)	133

"auxiliary phenomenon." However, as they pointed out, monoamine oxidase inhibitors also increase the brain levels of other centrally active amines, such as dopamine and norepinephrine. L-Tryptophan, on the other hand, increases the brain content of serotonin *without* simultaneously increasing the levels of catecholamines.

Perry (*15*) has also expressed doubt as to the importance of serotonin in cerebral development after observing that 7 days of subcutaneous phenylalanine administration, begun shortly after birth, lowered cerebral serotonin during this 7-day period of rapid development but left no residual deficits in "visual discrimination" when the rats were tested 5 to 7 weeks later. The absence of a behavioral alteration may be related to the duration of treatment or to the test instrument used, since Schalock and Klopfer (*16*), using different testing procedures, report permanent performance deficits when L-phenylalanine (3 g/kg) was administered daily by gavage (from birth to 60 days of age). Those investigators (*16*) and Hess *et al.* (*17*) suggest that certain behavioral assays may be more sensitive to impairments of the central nervous system in experimental phenylketonuria than other assays.

The work of Woolley and van der Hoeven (*18, 19*), which proposes a major role for serotonin in cerebral development, has received considerable attention. The major shortcoming in their studies relates to the absence of specific information on blood or tissue levels of phenylalanine or phenylketones, which prevents comparison of their biochemical conditions with those of other "phenylketonuric" preparations. These authors describe an irreversible "mental defect" in infant mice associated with poor T-maze performance and poor shock avoidance, which could be prevented by the administration of serotonin congeners (*18*). They could find no such "defect" in weanling mice fed diets containing 3 percent DL-phenylalanine and 3½ percent L-tyrosine. No blood phenylalanine values are given in either study and there is small likelihood that the concentrations of dietary phenylalanine used could have produced more than a transient rise in circulating L-phenylalanine levels.

In summary, diets high in phenylalanine or leucine are associated with poor water-maze performance and lowered brain serotonin, whereas diets high in tryptophan produce superior maze performance and supranormal levels of cerebral serotonin. Furthermore, the serotonin depletion and maze performance deficit associated with phenylketonuric rats can be more than compensated by adding 5 percent tryptophan to the phenylketogenic diet. This is compatible with a role for disturbed indole metabolism in the behavioral defect observed in phenylketonuric rats.

CHARLES M. MCKEAN
SAUL M. SCHANBERG*
NICHOLAS J. GIARMAN
Sonoma State Hospital,
Brain-Behavior Research Center,
Eldridge, California 95431, and
Department of Pharmacology,
Yale Medical School,
New Haven, Connecticut

References and Notes

1. A. Yuwiler and R. T. Louttit, *Science* **134**, 831 (1961).
2. C. M. McKean, S. M. Schanberg, N. J. Giarman, *ibid.* **137**, 604 (1962).
3. V. J. Polidora, D. E. Boggs, H. A. Waisman, *Proc. Soc. Exp. Biol. Med.* **113**, 817 (1963).
4. V. J. Polidora, R. F. Cunningham, H. A. Waisman, *J. Comp. Physiol. Psychol.* **61**, 436 (1966).
5. ———, *Science* **151**, 219 (1966).
6. J. H. Menkes, P. L. Hurst, J. M. Craig, *Pediatrics* **14**, 462 (1954).
7. B. Smith, and D. J. Prockop, *New Engl. J. Med.* **267**, 1338 (1962).
8. A. Yuwiler and E. Geller, *Nature* **208**, 83 (1965).
9. H. Green, S. M. Greenberg, R. W. Erickson, J. L. Sawyer, R. J. Ellison, *J. Pharmacol. Exp. Therap.* **136**, 174 (1962).
10. W. C. Biel, *J. Genet. Psychol.* **56**, 439 (1940).
11. D. F. Bogdanski, A. Pletscher, B. B. Brodie, S. Udenfriend *J. Pharmacol. Exp. Therap.* **117**, 82 (1956).
12. B. F. Skinner, *J. Genet. Psychol.* **9**, 3 (1933).
13. C. M. B. Pare, M. Sandler, R. S. Stace, *Lancet* **1957-I**, 551 (1957).
14. D. E. Boggs, R. S. de Ropp, C. M. McKean, *Federation Proc.* **23**, 146 (1964).
15. R. L. Perry, G. M. Ling, S. Hanse, L. MacDougall, *Proc Soc. Exp. Biol. Med.* **119**, 282 (1965).
16. R. L. Schalock and F. D. Klopfer, *Science* **155**, 1033 (1967).
17. S. M. Hess, E. C. Paulsen, S. A. Muller, P. Carlton, *Life Sci.* **5**, 927 (1966).
18. D. W. Woolley and T. van der Hoeven, *Science* **144**, 1593 (1964).
19. ———, *ibid.* **139**, 610 (1963).
20. Aided in part by grants NB-00940 from the National Institute of Neurological Diseases and Blindness and HD-01823 from the National Institute of Child Health and Human Development.
* Present address: National Institute of Mental Health, Bethesda, Md.

22 May 1967

Modulation of Elicited Behavior by a Fixed-Interval Schedule of Electric Shock Presentation

Abstract. *Responding elicited in the squirrel monkey by electric shocks presented every 60 seconds was gradually altered in temporal patterning, especially when the shock was also produced by responses under a 30-second fixed-interval schedule. The initially elicited pattern of maximal responding just after each shock was altered by the recurrent shock and by the added fixed-interval schedule to a pattern of maximal responding just before each shock. Most shocks were produced by responses and the response pattern was maintained for several months, but little responding occurred when shocks were omitted.*

A noxious stimulus, such as an intense electric shock, influences the behavior of animals differently, depending upon how the noxious stimulus is scheduled and the nature of the behavior preceding it. Responding can be engendered and maintained under conditions in which responses terminate or postpone electric shocks ("escape" or avoidance); ongoing responding can be suppressed under conditions in which responses are followed by electric shocks (punishment). We here describe the maintenance of responding, initially elicited by electric shock in a situation in which the only programmed consequence of responding was the scheduled delivery of electric shocks.

Typically, presenting a reinforcer after infrequently occurring responses will increase the rate of responding (*1*). When a known reinforcer follows specified responses that occur frequently, responding may be modulated more than changed in absolute level (*2*). Frequently occurring responses can also be modulated and maintained by the recurring presentation of reinforcers, such as food or electric shocks, without reference to behavior (adventitious rein-