

carboxylation step in the Calvin-Benson cycle (13, 14), resulting in high rates of carbon dioxide uptake under conditions of high irradiance, high temperature, and limited water supply (13). The inhibitory effect of atmospheric oxygen concentrations on net photosynthesis, present in species lacking the C<sub>4</sub> pathway, is absent in plants possessing this pathway. Since this inhibitory effect increases with increasing temperature, one might predict that its presence would be particularly detrimental to net photosynthesis in the Death Valley habitat of *T. oblongifolia*. Moreover, since C<sub>4</sub> photosynthesis evidently enables carbon dioxide of low concentrations in the leaf intercellular spaces to be utilized with a higher efficiency (lower "mesophyll resistance"), the efficiency of water use, that is, the amount of carbon dioxide fixed divided by the amount of water lost through transpiration, is considerably improved (13, 15). Whether higher plant species exist which do not possess the C<sub>4</sub> pathway and yet are capable of a photosynthetic performance at high temperatures equaling that of *T. oblongifolia* remains an interesting and important question.

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## Rapid Increase of Phenylethanolamine N-Methyltransferase by Environmental Stress in an Inbred Mouse Strain

**Abstract.** The activity of phenylethanolamine N-methyltransferase in mice of the C57Bl/Ka strain was determined after a 4°C stress. The enzyme activity increased 1.2-fold at the end of 3 hours and by 1.4-fold by the end of 6 hours of the stress. The results are in contrast to those from other species with intact animals in which the enzyme changes only after several days of chronic stress. Cycloheximide prevents the rise in enzyme activity, suggesting the increase may be due to protein synthesis. The increase may provide a model system for studying regulation of catecholamine biosynthetic enzymes.

Phenylethanolamine N-methyltransferase (PNMT) is the adrenal medullary enzyme involved in epinephrine formation (1). In the rat, this enzyme has been shown to respond to exogenous glucocorticoid administration along an extremely slow time course (2, 3). The half-life of the enzyme has been estimated to be 6 days in the hypophysectomized rat (3) and 20 to 50 days in the intact rat (4). Because of the extremely slow turnover of the enzyme in the rat, studies which have attempted to probe the relation between this enzyme and acute environmental stress situations have not been possible.

Studies from this laboratory have suggested that PNMT in an inbred mouse strain may be under both neuronal and glucocorticoid control (5). In one inbred mouse strain, a maximal PNMT response to exogenous glucocorticoid administration was seen 3 hours after injection of the drug (5). Because of the rapid response of the enzyme in these animals, it would appear that inbred mouse strains could serve as excellent models in which to study the relation between acute stress and PNMT activity.

We now report our studies in an inbred mouse strain of the C57Bl/Ka line. The mice (20 to 25 g, male) were obtained from the department of radiobiology, Stanford Medical Center. Animals were housed in a soundproof animal room maintained at constant temperature and humidity and were al-

lowed to acclimatize to these facilities and the light-dark cycle (LD, 13:11; lights on 7:00 a.m.) for 5 to 7 days before the study. Cold-stress exposure was carried out as follows: Animals were housed in groups of three in a litter-free stainless steel cage in a cold room maintained at 4°C. Animals were allowed free access to water and Purina Lab Chow during the period of cold exposure. Immediately after the cold exposure period was ended, animals were killed by cervical dislocation and their adrenals were removed. The glands were cleaned and homogenized as a pair in ice-cold 0.32M sucrose. Homogenates were centrifuged at 30,000g for 20 minutes. The PNMT activity was estimated by the modification of the method of Deguchi and Barchas (6) of the phenylethanolamine procedure originally described by Axelrod (1). Protein determinations were made according to the method of Lowry (7).

Table 1. Effects of cold exposure on adrenal PNMT activity in C57Bl/Ka mice. Each group contained nine animals; the values shown are the means and standard errors of those groups. One unit of PNMT activity is the formation of 1 nmole of N-methylphenylethanolamine per hour.

Treatment	PNMT activity (unit/mg protein)
Unstressed control	0.300 ± 0.005
3-hour cold stress	0.370 ± 0.024*
6-hour cold stress	0.417 ± 0.018†

\* P < .01 greater than unstressed control.

† P < .001 greater than unstressed control.

In the first experiment, mice were subjected to cold exposure for periods of 3 or 6 hours. The PNMT activity was significantly elevated (Table 1) at the end of 3 hours; this elevation was sustained at 6 hours.

To determine whether the rapid increase in PNMT activity represented an activation of existing enzyme or an induction of protein synthesis, the following experiment was performed. One hour before the initiation of cold exposure, animals were given 50 mg of cycloheximide per kilogram of body weight intraperitoneally; cycloheximide is an inhibitor of protein synthesis.

Cycloheximide dilutions were prepared in isotonic saline; control animals received an equivalent volume of saline. After 6 hours of cold exposure, the animals were killed, as stated before, and the PNMT in the adrenals was determined. The results (Fig. 1) confirm the rise in PNMT activity seen in the previous experiment (saline plus cold stress). However, cycloheximide completely blocked the stress-mediated rise in PNMT. These results suggest that stress brings about an increase in PNMT activity by stimulating protein synthesis. It remains to be elucidated whether stress induces the de novo synthesis of PNMT, or of some other protein which perhaps activates existing PNMT without increasing the absolute amount of the enzyme.

Our studies represent the first demonstration in any species that acute stress can cause a rapid induction of PNMT. The results also demonstrate species differences; since, in the rat, PNMT induction is important only in

response to long-term stress situations (3, 4, 8, 9), while in the mouse, PNMT may be important in the acute adrenal medullary stress response. The results raise the possibility that, in the mouse, a relatively rapid change in PNMT activity may be important in the ability of the organism to sustain output of epinephrine. The rapid rise of PNMT activity in the mouse could provide a valuable system for investigation of regulation of catecholamine biosynthesis (10).

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## Auditory Evoked Response: Meaningfulness of Stimuli and Interhemispheric Asymmetry

**Abstract.** *Interhemispheric asymmetries of different magnitudes were observed in human cortical auditory evoked responses to speech and sound-effect stimuli. The wave with peak asymmetry occurred 100 milliseconds after signal onset. The amount of asymmetry of the amplitude of this wave was related to the meaningfulness to the subject of the auditory stimulus rather than to the mere use of verbal versus nonverbal materials.*

Differences between the hemispheres in processing verbal as opposed to nonverbal stimuli have been demonstrated in man by clinical, pathological, pharmacological, and psychological techniques (1). The presence of specialized auditory areas in each hemisphere has been postulated (2), and an anatomical asymmetry between the speech area in the major hemisphere and its counterpart area in the minor hemisphere has been reported (3). These findings suggest that similar asymmetries between the two hemispheres might be detected by neurophysiological techniques.

We looked for interhemispheric asymmetries in the transcranially derived gross auditory evoked response (AER) in human subjects who were processing various verbal and nonverbal stimuli (4). However, the focus of this report is on the effect of variations in the meaningfulness of the stimulus materials, both verbal and nonverbal.

Using a rosette recording method (5) over the parietal, central, and temporal regions of two subjects, we established that the largest asymmetry of AER amplitude (W wave in Fig. 1) occurred about 100 msec after the stimulus. Measurements were made by comparing

two bipolar recordings,  $W_1-P_3$  and  $W_2-P_4$ . In these recordings,  $P_3$  and  $P_4$  were the left and right parietal locations as given by the standard 10-20 system (6), and  $W_1$  was defined as the center of a triangle made by 10-20-system locations  $P_3$ ,  $T_3$ , and  $T_5$ . This  $W_1$  placement was presumably close to Wernicke's area. Location  $W_2$  was similarly determined on the right hemisphere.

In some subjects, the interhemispheric asymmetry of the W waves consistently differed in magnitude as a function of the speech or nonspeech character of the stimuli.

We wondered whether these asymmetries in the gross AER were determined by the physical properties of speech and nonspeech sounds or by the significance attached by the subject to these different auditory stimuli. In other words, were these AER asymmetries attributable to the use made of the stimuli by the subject? To answer this question, we studied the AER obtained in the following experimental conditions.

Condition 1: indiscriminated words. Four monosyllabic words (back, tick, bake, and cook) recurred 110 times in all and were interspersed randomly

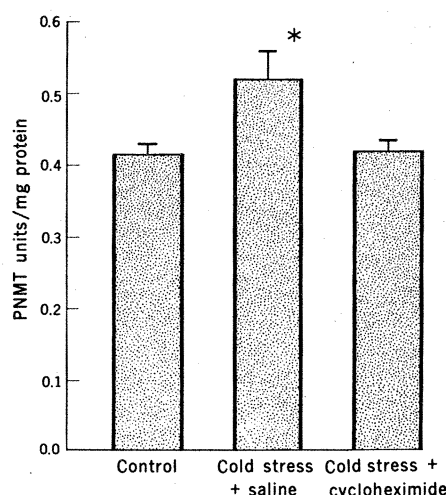


Fig. 1. Effects of cycloheximide on cold stress-mediated increase in PNMT. Animals were treated with saline or cycloheximide, cold-stressed, and killed 6 hours later. \* $P < .02$  greater than control.