evolution was severalfold higher than acetylene reduction because CO_2 fixation in these growing cultures was a metabolically more active reaction than nitrogen fixation. If the cultures were starved for nitrogen by flushing for 40 hours with a mixture of argon and CO_2 (99.7 : 0.3) or a mixture of argon, O_2, and CO_2 (79.7 : 20 : 0.3) in the light, oxygen evolution was sharply reduced (more than 90 percent), while hydrogen evolution, heterocyst frequency, and [as reported (*13*)] acetylene reduction increased. In these nitrogen-starved cultures, hydrogen evolution in the dark was severalfold higher than acetylene reduction in the dark, thus some of the hydrogen evolution appeared to be due to the reported reversible hydrogenase activity of *Anabaena cylindrica* (*14*).

Our data demonstrate that the heterocyst-vegetative cell system can be used to simultaneously produce hydrogen and oxygen from water and light energy. Although normally more oxygen than hydrogen is produced, manipulation of the cultures (as by nitrogen starvation) should allow the achievement of stoichiometric ratios. Although about a 10 percent conversion of solar energy into chemical energy can be theoretically achieved with photosynthetic processes, such an efficiency cannot yet be achieved or sustained in hydrogen production by heterocystous blue-green algae. However, we have shown the basic requirement for solar energy conversion: the decomposition of water into hydrogen and oxygen by light energy.

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Norepinephrine-Sensitive Adenylate Cyclases in Rat Brain: Relation to Behavior and Tyrosine Hydroxylase

Abstract. *Responses of norepinephrine-sensitive adenosine 3′,5′-monophosphate (cyclic AMP)–generating systems in combined midbrain-striatal slices of four rat strains correlate positively with spontaneous behavioral activity and negatively with levels of midbrain and striatal tyrosine hydroxylase. Responses of cerebral cortical norepinephrine-sensitive cyclic AMP systems correlate negatively with spontaneous behavioral activity and positively with midbrain and striatal tyrosine hydroxylase. Such correlations were not found with responses of the cyclic AMP–generating systems to isoproterenol, adenosine, veratridine, or of an adenosine and norepinephrine combination.*

The proposal of an interrelation between brain biogenic amines and behavior (*1*) and its subsequent elaboration (*2*) have been followed by numerous attempts to demonstrate a causal relation between steady state levels of brain biogenic amines and spontaneous behavioral activity in various rat and mouse strains. The results obtained from such studies (*3*) have been contradictory and unclear and emphasize our lack of understanding of the neurochemical correlates of behavior. Recently, however, an excellent negative correlation between spontaneous behaviorial activity and levels of tyrosine hydroxylase in midbrain ($r = -.94$) and striatum ($r = -.83$) was reported for several rat strains (*4*). It would, therefore, appear that the levels of tyrosine hydroxylase, the rate-limiting step in catecholamine biosynthesis (*5*), may provide a more appropriate measure of a functional pool of newly synthesized catecholamine, which is preferentially released during adrenergic neuronal activity (*6*) than does the steady state levels of catecholamines. The negative correlation between spontaneous motor activity and tyrosine hydroxylase activity was proposed (*4*) to indicate that low levels of transmitter biosynthesis are associated with a relatively high degree of functional activity of adrenergic receptor-mediated mechanisms and that this enhanced activity results in a high level of spontaneous behavioral activity. Conversely, high levels of transmitter biosynthesis were proposed to be associated with a relatively low degree of functional activity of adrenergic receptor-mediated mechanisms with a resultant low level of spontaneous behavioral activity. The functional activity of adrenergic receptor-mediated mechanisms could depend, of course, on at least two factors: (i) the number of activations of adrenergic receptors by "released" neurotransmitter norepinephrine, and (ii) the magnitude of the resultant biochemical signal from each activation.

Formation of adenosine 3′,5′-monophosphate (cyclic AMP) has been shown to be intimately associated with neuronal transmission in both the central (*7*) and peripheral (*8*) nervous systems. Accumulations of cyclic AMP have been demonstrated in brain tissue after stimulation by electrical means (*9*), depolarizing agents (*10*), adenosine (*11*), and biogenic amines (*12*). The proposed relation between tyrosine hydroxylase activity, receptor function, and spontaneous motor activity (*4*) and the possibility that an adenylate cyclase system is associated with adrenergic receptors if it is not the receptor itself (*13*) led us to investigate a possible association between the magnitudes of spontaneous motor and tyrosine hydroxylase activity and the norepinephrine-elicited accumulation of cyclic AMP in cerebral cortex and midbrain and striatum of various rat strains.

Adult male rat strains F344, ACI, and BUF were obtained from Microbiological Associates, Inc., Walkersville, Maryland, and adult male Sprague-Dawley rats were obtained from Taconic Farms, Germantown, New York. The rats were housed at our facilities for at least 1 week before use. Animals (175 to 225 g) were killed by decapitation, and cortical

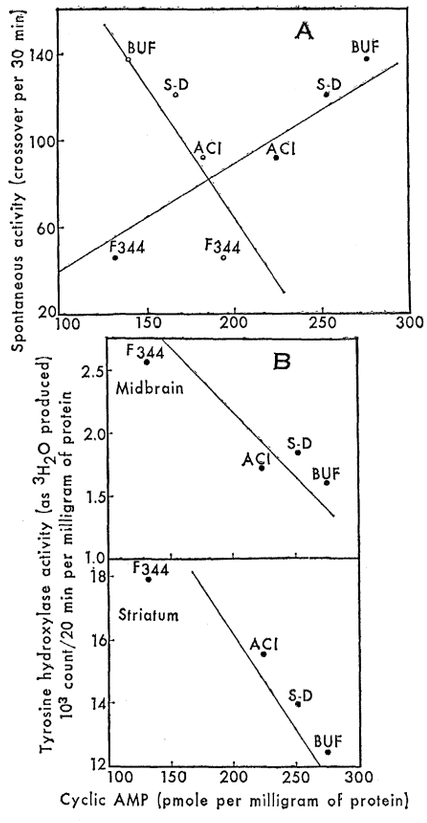

Fig. 1. (A) Correlation between norepinephrine-elicited accumulations of cyclic AMP in cortical slices (open circles) or in midbrain-striatal slices (closed circles) and spontaneous behavioral activity in four rat strains. Lines by regression analysis: open circles, $r = -.92$ ($P < .05$); closed circles, $r = .99$ ($P < .001$). (B) Correlations between norepinephrine-elicited accumulations of cyclic AMP in midbrain-striatal slices and levels of tyrosine hydroxylase in midbrain (upper panel) and striatum (lower panel). Data on accumulation of cyclic AMP are from Table 1. Data on spontaneous behavioral activity and tyrosine hydroxylase levels in BUF, Sprague-Dawley (*S-D*), ACI, and F344 strains of rats are from Segal *et al.* (*4*). Lines by regression analysis: upper panel, $r = -.95$ ($P < .05$); lower panel $r = -.98$ ($P < .01$)

slices were prepared (*14*). Midbrain and striatum, after being dissected (*15*), were combined, minced briefly with a razor blade, and then chopped on a McIlwain tissue chopper as described for cerebral cortex (*14*). The time from decapitation to incubation was usually less than 20 minutes. Chopped tissue was incubated as described (*14*) in a Krebs-Ringer bicarbonate glucose medium for 15 minutes, then transferred to medium containing 17 μM adenine for 40 minutes, followed by two washings with medium and incubation for 10 minutes. Slices were collected on fine nylon mesh, divided into portions, transferred to beakers containing the appropriate test agents in 10 ml of medium, and then incubated for 9 to 15 minutes. Tissue was then collected on nylon mesh and rapidly transferred to homogenizers containing 1 ml of cold 8 percent trichloroacetic acid; the tissues were homogenized, and cyclic AMP was determined (*16*). Protein was determined by the Miller modification (*17*) of the Lowry method (*18*). Bovine serum albumin was used as the protein standard.

The four strains of rats used were strains whose spontaneous motor activity (quadrant crossing under standardized conditions) has been shown (*4*) to be inversely correlated with tyrosine hydroxylase activity in both midbrain and striatum. Examination of the norepinephrine-elicited accumulation of cyclic AMP in the cerebral cortex of these rats revealed a negative correlation with spontaneous behavioral activity ($r = -.92$; $P < .05$), while a positive correlation pertained with data on cyclic AMP accumulation in midbrain-striatum ($r = .99$; $P < .001$) (Fig. 1A). A negative correlation was found between the norepinephrine-elicited accumulation of cyclic AMP in slices from midbrain-striatum and tyrosine hydroxylase activties in midbrain ($r = -.95$; $P < .05$) and striatum ($r = -.98$; $P < .01$) (Fig. 1B). Positive correlations were obtained between the norepinephrine-elicited accumulation of cyclic AMP in cerebral cortex and activties of tyrosine hydroxylase in midbrain ($r = .75$; not significant) and striatum ($r = .96$; $P < .05$). In addition, a negative correlation was found between the norepinephrine-elicited accumulation of cyclic AMP in cerebral cortex and that elicited in midbrain-striatum ($r = -.88$; $P < .08$).

These correlations appear to obtain only after stimulation of brain tissue with norepinephrine. The accumulation of cyclic AMP elicited by isoproterenol, veratridine, adenosine, or adenosine-norepinephrine combinations (Table 1) did not correlate with either spontaneous behavioral activity or tyrosine hydroxylase activity. The lack of correlation between tyrosine hydroxylase activity or spontaneous behavioral activity and the accumulation of cyclic AMP elicited by isoproterenol, a classic β-adrenergic agent in rat brain (*19*), suggests that an α-adrenergic function may be the common link between spontaneous behavioral activity, tyrosine hydroxylase activity, and catecholamine-elicited accumulation of cyclic AMP in the brain.

Undoubtedly, the final manifestation of spontaneous behavioral activity involves a complex integration of neuronal circuitry, involving the function of adrenergic and other systems. However, correlations of low activities of tyrosine hydroxylase with high spontaneous behavioral activity led Segal *et al.* (*4*) to propose that, under conditions of decreased synthesis and

Table 1. Accumulation of cyclic AMP in cortex and midbrain-striatum of different rat strains. Tissue was stimulated with agents for 9 to 15 minutes. The concentration of the agents was $10^{-4}M$, except for veratridine which was $8 \times 10^{-5}M$. Each value represents the mean ± standard error of the mean of three or four experiments, each experiment with the pooled tissue from two rats.

Agent	Cyclic AMP (picomoles per milligram of protein)							
	BUF		Sprague-Dawley		ACI		F-344	
	Cortex	Midbrain-striatum	Cortex	Midbrain-striatum	Cortex	Midbrain-striatum	Cortex	Midbrain-striatum
None	41 ± 6	43 ± 5	39 ± 4	64 ± 13	39 ± 12	42 ± 6	40 ± 4	61 ± 8
Norepinephrine	140 ± 32	275 ± 36	166 ± 37	252 ± 13	182 ± 35	223 ± 82	194 ± 30	132 ± 33
Isoproterenol	90 ± 18	82 ± 5	82 ± 12	112 ± 13	68 ± 11	56 ± 10	115 ± 11	107 ± 22
Adenosine	289 ± 43	283 ± 55	192 ± 22	402 ± 108	234 ± 81	232 ± 79	282 ± 55	306 ± 81
Adenosine-norepinephrine	789 ± 71		937 ± 94		579 ± 192		768 ± 88	
Veratridine	397 ± 45		330 ± 38		382 ± 80		393 ± 107	

hence release of catecholamines, adrenergic receptor responses are compensatorily increased, resulting in a higher level of spontaneous behavioral activity. Our observation of a positive correlation between spontaneous motor activity and the responses of the norepinephrine-sensitive cyclic AMP–generating system in midbrain-striatum is consistent with the hypothesis that the relevant adrenergic receptor response is a norepinephrine-elicited accumulation of cyclic AMP in midbrain-striatum. The negative correlation obtained between spontaneous behavioral activity and norepinephrine-induced accumulation of cyclic AMP in cerebral cortex would at first appear to be inconsistent with the above hypothesis. However, stimulation of adrenergic "receptors" in the cerebral cortex, manifested as an increased accumulation of cyclic AMP, might in this brain region be associated with inhibitory (*7*) rather than excitatory pathways. A high "receptor" activity could then result in a lowered level of spontaneous behavioral activity.

Our observation that relatively high accumulations of cyclic AMP elicited in midbrain-striatum by norepinephrine are associated with low levels of tyrosine hydroxylase in this brain region supports the proposal (*4*) that low levels of tyrosine hydroxylase are associated with a relatively high functional response of adrenergic receptors. At present, it is uncertain whether a similar correlation obtains between tyrosine hydroxylase activity and the magnitude of norepinephrine-elicited accumulation of cyclic AMP in cerebral cortex. Comparison of tyrosine hydroxylase activity in midbrain and striatum with responses of the cortical norepinephrine-sensitive cyclic AMP–generating system reveals, however, a negative correlation. One possible explanation is that the enhanced activity of midbrain and striatal receptor systems results in compensatory reductions in responses of cortical systems. Certainly, the magnitude of norepinephrine-elicited accumulations of cyclic AMP in cortical and midbrain and striatal slices have a negative correlation. These interrelations between the activity of cyclic AMP–generating systems in different brain regions and adrenergic function in brain require further investigation.

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Dopamine Nerve Terminals in the Rat Limbic Cortex: Aspects of the Dopamine Hypothesis of Schizophrenia

Abstract. *The existence of cortical dopamine nerve terminals is demonstrated with a highly sensitive modification of the Falck-Hillarp fluorescence technique. This confirms previous biochemical reports of high dopamine levels in the cortex. The histochemistry reveals that the distribution is regional and confined to the limbic cortex.*

Increasing interest has been focused on central catecholamines (CA) as a possible biochemical correlate of schizophrenia (*1, 2*). This hypothesis is mainly based on clinical experience with and basic research on certain types of adrenergic drugs (*3*). Thus, amphetamine, which is known to release endogenous CA (*4*), in small doses aggravates the symptoms of schizophrenic patients and in large doses evokes a psychosis clinically indistinguishable from acute paranoid schizophrenia [see references cited by Snyder (*3*)]. It has been proposed by Kety (*5*) that this state may be considered a "model" schizophrenia. On the other hand, a good correlation between the beneficial effects of certain phenothiazines in the treatment of schizophrenia and the effects of these drugs on monoamines in the central nervous system has been observed (*2*). As originally suggested by Carlsson and Lindqvist (*6*), phenothiazines may cause a blockade of central CA, especially dopamine (DA), receptors. Thus, two groups of drugs known to affect transmission at CA synapses in opposite ways seem to have mental effects in man which may be interpreted as antagonistic.

The best correlation, as far as monoamines are concerned, seem to be between DA and schizophrenia (*3*). However, as judged from fluorescence histochemical studies (*7*) and also biochemistry (*8*), DA was until recently assumed to be localized almost exclusively in the basal ganglia, the subcortical limbic system, and the hypothalamus, areas which do not immediately seem related to some of the classical symptoms in schizophrenia. The elegant combined biochemical and lesion experiments of Thierry *et al.* (*9*) demonstrate comparatively high levels of DA in the rat cortex and give convincing evidence that this DA is present in nerves probably not identical with the cortical noradrenaline (NA) neurons described in previous histochemical studies (*10*). These results have prompted us to reinvestigate the existence of cortical DA neurons with some recent, highly sensitive modifications of the Falck-Hillarp technique (*11*) in combination with drug models designed to visualize selectively DA neurons (*12*).

Different cortical brain areas of three groups of male albino rats (Sprague-Dawley, 150 to 200 g) were studied. In the treated groups drugs were administered intraperitoneally, at the specified times before the animals were